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ON THE CARBON: NITROGEN (C/N) RATIO IN THE URINE OF RATS DEPRIVED OF ONE OR BOTH FACTORS OF THE VITAMIN B COMPLEX

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In recent years Bickel and his school (1, 2, 10,) have published a series of important papers dealing with the assimilation and excretion of carbon and nitrogen in the various pathological conditions involved in human and experimental avitaminoses. They found that when dogs were subjected to a multiple vitamin deficiency (the diet consisting of washed polished rice, wheat protein, glucose, lard and salts and thus containing probably only traces of vitamins A, B, C, and D) there was a marked increase of the carbon excreted in the urine as compared with the excreted nitrogen, leading to an increase of the ratio C/N sometimes above unity. When vitamins in the form of butter, yeast and lemon juce were added to the above diet the C/N ratio was about .74. The increase of the C/N ratio is attributed by these investigators to an excretion in the urine of partially oxidized carbon compounds and this phenomenon has been termed by them desoxydative carbonuria.

Kauffmann-Cosla and Roche (4) estimated the excretion of lactic acid and acetaldehyde as well as the C/N ratio in the urine of two dogs subjected to the multiply deficient diet used by Bickel. They noted an increase of the C/N ratio from a normal average of .74 to 1.125 (average for ten days) and also an increased output of lactic acid and acetaldehyde which, however, accounted for only a small part of the total increase of the urinary carbon. They point out the possible rôle of vitamin B deficiency in connection with the dyscarbonuria but owing to the complex nature of the deficiency to which the animals were subjected, they cautiously abstained from any definite conclusions. A year later, Roche (8) using the same technique was able to demonstrate that whereas the addition

of lemon juice and butter, alone or combined, to the experimental ration of Bickel did not cause a decrease of a high C/N ratio, this was brought back to normal by the inclusion of yeast in the diet. About the same time certain experiments carried out in this laboratory made it necessary to investigate the question of food assimilation in the absence of one or both factors of the vitamin B complex. As a first step it was decided to estimate the C/N ratio in the urine of a larger group of rats receiving a diet, deficient either in the heat-stable or heat-labile component of vitamin B or devoid of the whole complex. The methods applied to this study were similar to those successfully used by Kon and Drummond (5) in their study of vitamin B deficiency in pigeons, and involving the use of individual controls receiving a complete diet restricted in amount to the quantities ingested day by day by their mates kept on a deficient diet. This arrangement makes it possible to differentiate changes due to starvation incident to vitamin B deficiency from those due to the lack of the vitamin itself. Diet 540 of this laboratory was used as the basic ration. It has been repeatedly shown to be remarkably free of the vitamin B complex and on the other hand its simple composition facilitates the interpretation of results.

TABLE I
CARBON : NITROGEN RATIO

Period	Group I			Group II			Group III			
	Experimental Group A	Control Group A	Experimental Group B	Control Group B	Experimental Group A	Control Group A	Experimental Group B	Control Group B	Experimental Group A	Control Group A	Experimental Group B	Control Group B
1	0.78	0.72	0.75	0.72	0.85	0.76	0.86	0.68	0.64	0.76	0.70	0.75
2	0.56	0.55	0.58	0.58	0.70	0.60	0.67	0.60	0.63	0.62	0.69	0.62
3	0.59	0.53	0.57	0.57	0.70	0.56	0.66	0.55	0.55	0.54	0.66	0.60
4	0.69	0.53	0.65	0.58	0.70	0.54	0.68	0.56	0.61	0.55	0.53	0.57
5					0.68	0.57	0.69	0.59	0.63	0.58	0.65	0.67
6					0.71	0.58	0.75	0.62			0.71	0.59
7					0.80	0.55	0.75	0.68				
8					0.80	0.58	0.77	0.61				

When the present investigation was under way Roche (9) reported results dealing with C/N ratio in the urine of starving and vitamin B deficient rats.

Experimental—36 female rats were used in this study. They were weaned when three weeks old and then placed on the standard stock diet (Diet I) of this laboratory. The diet has the following composition: Whole wheat 67.5 per cent, casein 15, whole milk powder 10, sodium chloride 1, calcium carbonate 1.5, butter fat 5. When 24 or 25 days old, the rats were split into pairs of litter mates of approximately the same weight and three groups of twelve were formed, each consisting of six pairs, one member of the pair acting as individual control for its litter mate. The rats were transferred to diet 540 (casein extracted with acidulated water 25, commercial cane sugar 75, salts (Mc Collum 185) 4) and placed in groups of three in metabolism cages. The round cages of eight inches diameter were placed in a rack each on top of a large glass funnel. The cages had a wire bottom of 1/2 inch mesh through which urine and feces dropped through the funnel, where the feces were stopped by a wire screen and the urine filtered through a pad of glass wool into cylindrical vessels of 50 cc. capacity placed under each funnel. Each cage was supplied with an inverted narrow necked glass bulb containing distilled water. A separate set of individual screened cages was used for feeding the rats for two reasons: firstly, the nature of the diet used containing a water-soluble carbohydrate made it imperative to prevent any contamination of the urine, which obviously would completely vitiate the results, and unfortunately a perfect feeding device totally preventing scattering of the food is yet to be found. Secondly, in order to measure the food intake of every rat separately,the diet was offered in small heavy cups closely fitting into metal cylinders closed at the top and having a hole in the center large enough to admit the head of a rat, but preventing him from scratching the food out with his paws. The rats were given daily, separately from their diet, two drops of wheat germ oil[1] and two drops of cod-liver oil[2] as a source of vitamins E and of A and D respectively. All the control animals were also given 500 mg. of yeast.[3] Six rats of the first group were used for a study of total deficiency of the vitamin B complex and received no other addition to their food than the fat-soluble vitamins. Half of the second group was given

[1] Wheat germ oil prepared by extracting fresh whole wheat germ with ether at room temperature.

[2] Patch's cod-liver oil.

[3] Northwestern yeast—Group I received 0.3 cc. of an extract of brewer's yeast prepared by the method of Osborne and Wakeman.

daily 3 cc. of a 90 per cent alcoholic extract of rice polishings equivalent to 3 grams of rice polishings. This preparation was found in this laboratory to contain a sufficient amount of the heat-labile factor but only traces of the heat-stable (pellagra-preventing) factor. Half of the third group was given 700 mg. autoclaved yeast[4] daily, thus receiving enough of the heat-stable but no heat-labile factor. On the first day of the experiment all the rats were allowed free access to the food for a period of four hours. They were then transferred to the metabolism cages and the food intake of the experimental animals was measured. The food left in the food cups of the control rats was discarded and an amount equal to that eaten by their mates was weighed out. In the second group, the controls were given .25 gram less than the other member of the pair, in order to counterbalance the additional .5 gram of yeast allotted to the control. The next day the rats were transferred from the metabolism cages to the individual feeding cages when the controls were offered the food weighed out the day before, while their mates were, as previously, offered the diet *ad libitum*. This process was from now on repeated every day. After a few days, the time allowed for feeding was reduced from four hours to one hour and a half, the rats spending 22½ hours in the metabolism cages. In the beginning, the controls sometimes left a part of their allotment. This was then added to the food weighed out for the following day. Soon, however, the consumption became quantitative. Each day the samples of urine collected from every cage were poured into larger containers and stored in a refrigerator at 0°C. The cages, screens, funnels and containers were then washed with hot water, wiped dry and three drops of a 1 per cent solution of $Hg(CN)_2$ were added to each collecting vessel in order to prevent any decomposition of urine during the 24-hour period of collection. The urine was analysed in weekly samples, the carbon being estimated by the excellent micro-method of Nicloux (6, 7), and the nitrogen by the macro-Kjeldahl method. All the analyses were made in duplicate. Owing to the necessity of feeding the animals out of the metabolism cages no quantitative collection of urine was possible. For this reason no particular care was exercised in collecting the daily samples and any urine drying on the sides of the funnels was discarded. As, however, all the operations were carried exactly alike for both the experimental animals and the controls, and as, furthermore, every analysed sample represented the collection of three rats over a period of one week, it is felt that the results obtained are as reliable as can be reasonably expected in the biological world. In the case of an experimental

[4] Fleishmann's yeast—autoclaved at 25 lbs. pressure for 5 hours.

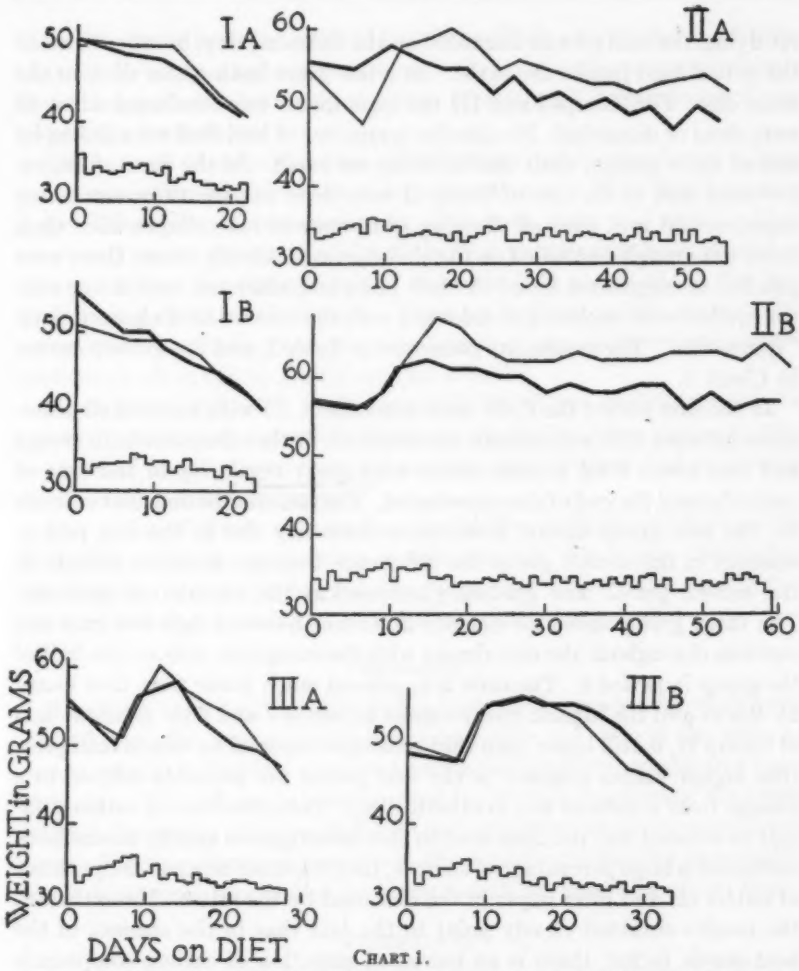

CHART 1.

Curves depicting the growth of rats used for the determination of the *C/N* ratio in the urine. In each case a curve represents the growth of three animals, the heavy line the experimental animals, and the light line their litter mate controls. The lowest curve indicates the average food intake for each member of the group. All animals received two drops of cod liver oil and three drops of wheat germ oil daily.

Group I. A and B—Experimental animals receiving Diet 540 *ad iibitum*, Control animals receiving Diet 540 (limited) and 0.3 cc. of an extract of brewer's yeast prepared by the method of Osborne and Wakeman.

Group II. A and B—Experimental animals receiving Diet 540 *ad libitum* and 3 cc. of a 90 per cent alcoholic extract of rice polish. Control animals receiving Diet 540 (limited) and 500 mg. of yeast.

Group III. A and B—Experimental animals receiving Diet 540 *ad libitum* and 700 mg. autoclaved yeast. Control animals receiving diet 540 (limited) and 500 mg. of yeast.

rat dying, the control was discarded on the following day, in order to make the actual food intake the same. In a few cases both mates died on the same day. For Groups I and III the experiment was concluded when all were dead or discarded. No definite symptoms of beri-beri were shown by rats of these groups, their decline being too rapid. At the close of the experiment half of the rats of Group II were dead and the three remaining experimental rats were all showing symptoms of rat pellagra viz: their coats were rough and soiled on the abdomen with bloody urine; there were patches of coagulated blood on their paws and vibrissae, and in one case the eyelids were swollen and reddened with the formation of characteristic "spectacles." The results are presented in Table I, and the growth curves in Chart 1.

In the first period the C/N ratio runs about .75 with no marked differences between deficient animals and controls. It then drops for both groups and this lower level is maintained with great regularity in the case of controls until the end of the experiment. The ratio for the deficient animals for the first group shows, however, a secondary rise in the last period, whereas in the second group the difference becomes manifest already in the second period and gradually increases as the experiment proceeds. The third group shows no definite difference between deficient rats and controls throughout the experiment with the exception only of one-half of the group in period 6. The ratio is in general much lower than that found by Roche and the highest one, attained in period 7 and 8 by deficient rats of Group II, is still lower than that considered normal by this investigator (the higher values attained in the first period are probably related to a change from a natural to a synthetic diet). This difference is rather difficult to account for; the diets used in this investigation and by Roche both contained a large percentage of sucrose, the difference being in the presence of butter fat and filter paper in the diet used by the latter. Nevertheless, the results obtained clearly point to the fact that in the absence of the heat-stable factor, there is an increased excretion of carbon compounds as compared with nitrogenous bodies in the urine. It can also be seen that whereas the growth curves of deficient animals and controls in Groups I and III are almost identical, the controls are definitely heavier in the second group. It seems safe to conclude that the heat-stable factor is in some way linked with the metabolic processes of the organism, whereas it is not apparent from this work that this is the case with the heat-labile moiety of the vitamin B complex.

The nature of the metabolic disorder following withdrawal of the vitamin B complex from a synthetic ration remains at present unknown, but

it would appear from the work of Kauffman-Cosla and Roche and Roche, that the carbonaceous bodies excreted in the urine in the course of vitamin B deficiency do not belong to the class of intermediate products of carbohydrate breakdown.

Collazo and Munilla (3) have recently reported having found a large increase of ether-soluble substances in the urine of a 20 kg. dog kept on a polydeficient diet (autoclaved meat, rice, lard, sugar and salts). They recovered up to 20 grams of "fat" from a twenty-four hour sample of urine and state that the addition of vitamins A, C, and D to the diet had no lowering effect on the lipuria. They are inclined to believe that this excretion of fatty substances might be largely responsible for the increased carbonuria observed by Bickel and his school.

Further insight in this highly important problem would probably be gained by means of a complete metabolic study involving a quantitative analysis of ingested and excreted carbon and nitrogen and a parallel study of the gaseous exchange.

The writer wishes to express his appreciation to Professor Herbert M. Evans for aid in the prosecution of this research. He is also indebted to the Rockefeller Foundation for the grant of a Fellowship, during the tenure of which the work here reported was carried out.

Summary

Rats kept on a diet deficient in the heat-stable component of the vitamin B complex show an increased carbon: nitrogen ratio in the urine as compared with individual controls receiving equivalent amounts of a complete diet. This difference is less marked for animals deprived of the whole vitamin B complex and is negligible in the case of rats receiving autoclaved yeast. We may, therefore, entertain the hypothesis that the heat-stable factor is in some way linked with the metabolic processes of the body.

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2. Bickel, *Med. Klinik* 1925 No. 29.
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4. Kauffman, Cosla and Roche, *Compt. Rend. Soc. Biol.* 1927, XCVII, 807.
5. Kon and Drummond, *Biochem. Jour.* 1927, XXI, 632.
6. Nicloux, *Bull. Soc. Chim. Biol.* 1927, IX, 639.
7. Nicloux, *Compt. Rend. Soc. Biol.* 1928, XCVIII, 1225.
8. Roche, *Compt. Rend. Soc. Biol.* 1928, XCIX, 589.
9. Roche, *Compt. Rend. Soc. Biol.* 1928, XCIX, 671.
10. Shimizu, *Biochem. Zeitschr.* 1924, CLIII, 424.

THE MEASUREMENT OF THE BASAL HEAT PRODUCTION OF PIGEONS

I. INSTRUMENTAL TECHNIQUE

By

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Introduction

Statement of Problems

THE colony of birds on which this study is based is peculiarly fitted for an examination of the relation which metabolism bears to sex, race and hybridity. On the other hand, a thorough survey of the basal metabolism of these birds is required for the further elucidation of numerous studies conducted during the past 17 years by Riddle and coworkers on sex, race and hybridity within this colony. These two considerations have decided the nature and scope of the problems which we are investigating in these animals.

At present no question is more important in the physiology and genetics of sex than that of the relation which metabolism bears to sex. Very few satisfactory studies utilizing definite measures of heat-production or of the gaseous metabolism as the criterion of metabolic rate, have been made on the two sexes. Benedict and Emmes[1], Gephart and Du Bois[2], and Benedict and Talbot[3] have conducted such investigations on the human. In individuals weighing more than 11 kilos a sex difference was found, the males having the higher metabolic rate. Less direct and less definite measures have been made on a number of animals; the more adequate of these studies, according to a recent review of them by Riddle,[4] usually or always

[1] Benedict, F. G. and L. E. Emmes, A comparison of the basal metabolism of normal men and women. *Jour. Biol. Chem.* 1915, xx, 253; *Ibid., Proc. Nat. Acad. Sci.* 1915, i, 104.

[2] Gephart, F. C. and E. F. Du Bois., Clinical Calorimetry. *Arch. Int. Med.*, 1916, xvii, 902.

[3] Benedict, F. G. and F. B. Talbot, Metabolism and growth from birth to puberty. *Carn. Inst. Wash. Publ.*, No. 302, 1921.

[4] Riddle, O., Proofs and implications of complete sex-transformation in animals. *Verh. 1st Internat. Kong. für Sexualforsch.*, 1927, i, 193.

indicate a higher metabolism in the males. Some such indirect measures on doves and pigeons have earlier been made by Riddle and here also the results indicated a higher metabolic rate in males in both the egg stage[5] and the embryonic stage.[6] One of the ultimate purposes of this series of collaborative studies—among which the present investigation is the introductory—is to learn whether a sex differential exists in the basal metabolism of these birds at two later stages, namely in the young and in the adults. When this task is completed we shall have, in both doves and pigeons, unique knowledge of the relation which metabolism bears to sex in all life stages from egg to adult.

Comparative studies on the basal metabolism of different races of mankind are complicated by a number of factors (climate, diet, habits) which are difficult to control. In very distinct and long established races of pigeons, however, all or most such factors would seem to be eliminable or reducible to a common level. Of still greater interest to our program of research is the circumstance that certain races of doves have been established on the basis of hereditary differences in thyroid size. At the Station for Experimental Evolution one of us now has a few races of doves characterized by large thyroid glands, and other races characterized by small thyroids. Since the thyroid gland exercises so pronounced an influence on heat production, these "thyroid races" are specially suitable for studies on metabolism. Moreover, since the *size* of the thyroid is of itself not declarative of a high or low rate of functioning, the metabolism measurements are necessary for a determination of the real meaning of large or small thyroid size in each of these races. Altogether 13 races of common pigeons, and 24 races of ring doves, are available for these racial studies.

Doves and pigeons offer an unrivalled opportunity for the study of the metabolism of hybrids. It is in this group of animals alone that adult hybrids can be obtained from the crossing of species which belong to different zoological families. It also happens that in these hybrids, from parents so dissimilar, the sex ratio is greatly modified, only males being produced from the widest crosses. Here again the measurements included in our program of study have another opportunity to throw light upon any special relation which metabolism may bear to sex. Similarly, in these hybrids it will be possible to learn whether a different rate of heat production is at all concerned in the "increased vigor" of hybrids.

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[5] Riddle, O., Sex control and known correlations in pigeons. *Amer. Nat.*, 1916, L, 385.

[6] Riddle, O., Differential survival of male and female embryos in increased and decreased pressures of oxygen. *Proc. Soc. Exp. Biol. and Med.*, 1920, XVIII, 88.

It remains to note some additional earlier studies on special aspects of the metabolism of these doves and pigeons which provide a valuable background for the present investigation. Besides the studies on sex, to which reference is made above, a series of studies by Riddle and coworkers has dealt with seasonal changes in several of the endocrine organs—ovaries, testes, thyroids, parathyroids, liver, and spleen—of these birds. Those results should assist an understanding of changes in basal metabolism, or provide valuable considerations relative to the absence of such changes. Still another group of studies has shown that one stage of reproduction, namely ovulation, is accompanied by extraordinary changes in the blood of these birds; marked increases of sugar, calcium, fat and phosphorus have been demonstrated.[7] Whether these notable changes in the blood are accompanied by an increased or by a decreased basal metabolism is a special problem that will ultimately be resolved by this investigation.

In order to obtain basal and comparable measurements for a solution of any of the above-mentioned primary problems it has, of course, been obligatory to resolve a number of questions concerning the special technique required in work with these animals. Such problems have presented themselves in very considerable number and complexity. They may be listed as follows:

1.—Apparatus suitable for work with these animals.
2.—The necessary length of the fasting period.
3.—The effects of measurements at different temperatures.
4.—The effects of different temperatures during the 24 hours preceding measurement.
5.—The effects of confinement in larger or smaller cages preceding the measurement.
6.—Differences of measurement made by day and by night.
7.—The effects of light and darkness during measurement on the bird's activity and heat production.
8.—Effects of repeating measurements on the same bird at short intervals.
9.—Effects of age of the bird.

The two present communications must deal almost exclusively with the development of suitable apparatus (Part I), and with our studies of this lengthy list of factors or conditions—the necessary physiological technique (Part II).

The Nutrition Laboratory, entering into a comprehensive study of

[7] Riddle, O., Metabolic changes in the body of female pigeons at ovulation. *Proc. Amer. Phil. Soc.*, 1927, LXVI, 497.

comparative physiology with special reference to metabolism, has included in its series of studies, in addition to those on man, metabolism measurements on various animals ranging in size from the 600-kg. steer to the immature albino rat. Thus, not only have the usual laboratory animals such as the dog, the rabbit, the guinea pig, and the goose been considered, but special studies have been made of wild birds under aviary life and of many of the larger cold-blooded animals, such as snakes, tortoises and the great lizards. The results on the metabolism of wild birds have been published and the defects in this study clearly pointed out.[8]

The various doves and pigeons are typical of birds with high cell temperature (106° F.) and are well adapted for comparative metabolism studies. The present writers have therefore found it mutually advantageous to conduct a series of cooperative studies on this material at the Carnegie Station for Experimental Evolution, where Director C. B. Davenport has kindly assigned the necessary laboratory space to our work.

Literature

Metabolism measurements on various types of pigeons and doves have been made in considerable number. Delaroche,[9] in 1813, was the first to record observations on pigeons, followed a decade later by Allen and Pepys.[10] Dulong[11] in 1841 published data on two pigeons and Boussingault[12] in 1844 reported a number of measurements on turtle-doves. Almost simultaneously Letellier[13] included a turtle-dove in his study of the influence of extreme differences in temperature upon metabolism, and Regnault and Reiset[14] included pigeons among the animals they studied. The next year Lehmann,[15] laying further emphasis upon the effect of environmental temperature and of different degrees of humidity, made observations upon a rock pigeon. Bert,[16] in 1870, made experiments with a pigeon and other animals, primarily to determine the influence of size upon metabolism. Corin and Van Beneden[17] employed a respiration

[8] Benedict, F. G. and E. L. Fox, Bicentenary Number *Proc. Amer. Philos. Soc.*, 1927, LXVI, 511.

[9] Delaroche, M., *Journ. de Phys., de Chim., d'histoire natur.*, 1813, LXXVII, 5. (Cited by Sjoström, *Skand. Arch.*, XXX, 3).

[10] Allen and Pepys. 1829. (Cited by Soum, *Dissert*, Paris, 1896).

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[15] Lehmann, C. G., *Abhandl. bei Begründung der K. sächs. Ges. d. Wiss.*, Leipzig, 1846, 463. (Cited by Lehmann, Lehrb. d. Physiol. Chem., 1853, III, 303).

[16] Bert, P., Leçons sur la physiologie comparée de la respiration, 1870, 502.

[17] Corin, G. and A. Van Beneden, Travaux du Laboratoire de Léon Fredericq, 1885–86, I, 101; VII, 265.

apparatus on the Pettenkofer principle, and also a modified d'Arsonval air calorimeter, studying chiefly pigeons deprived of cerebral hemispheres but also a few normal controls. In 1890 Richet [18] presented data with regard to the common pigeon, and during the following twenty-five years investigations on this type of bird were published by Chapman and Brubaker,[19] Pembrey,[20] Soum,[21] Falloise,[22] Voit,[23] Buchanan,[24] and Ramoino.[25] Since 1920, especially, the data available for pigeons and doves have been steadily increased by the observations of Novaro,[26] Abderhalden,[27] Caridroit,[28] Magne and Simonnet,[29] Giaja,[30] Farmer and Redenbaugh,[31] Sierens and Noyons,[32] Moltschanowa,[33] Messerle,[34] Kartascheffsky,[35] Groebbels,[36] Terroine and Trautman,[37] Chahovitch,[38] and Mazko and Moltschanowa.[39]

Many of the observations on pigeons and doves represent chiefly studies of pharmacological problems and of deficiency diseases, particularly avitaminosis. The literature dealing with these special problems is so extensive that only those investigations are here cited which include observations on normal birds. Undoubtedly these birds made admirable controls and the values obtained with them served their specific purpose in the specially designed experiments. But we believe that the findings which will be set forth later in this paper show that some of the conditions

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[34] Messerle, N., *Arch. f. d. ges. Physiol.*, 1926, CCXIII, 419.

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[36] Groebbels, F., Arch. f. d. ges. Physiol., 1927, CCXVIII, 98; Biol. Zentralbl., 1927, XLVII, 344.

[37] Terroine, E. F. and S. Trautman, *Ann. de physiol.*, 1927, III, 422.

[38] Chahovitch, X., *Compt. Rend.*, 1927, XCVI, 298; *Arch. intern. Phys.*, 1926, XXVII, 150.

[39] Mazko, S. N. and O. Moltschanowa. *Journal exptl. biol. med.* (Russian), 1928, IX, 37.

prerequisite for basal metabolism measurements have not been recognized and utilized in earlier work with these birds.

The Respiration Apparatus Used

A special closed-circuit respiration chamber was constructed at the Nutrition Laboratory, first, for use with a single ring dove. This same type of chamber was subsequently modified so that it was large enough to include a common pigeon, and two or three copies of this type of apparatus were constructed; thus either the smaller bird (the dove, average weight 150 grams) or the larger common pigeon (weight 300-550 grams) could be used. Later an open-circuit apparatus for both doves and pigeons was devised, and finally, a special chamber was made so that a group of ten common pigeons or ten ring doves could be simultaneously studied.

The Closed-Circuit Apparatus

The respiration apparatus used for the individual bird is so designed as to permit measurements not only of the carbon dioxide production but likewise of the oxygen consumption. It is based upon the closed-circuit principle originally used by Regnault and Reiset. With the exception of the metabolism chamber which is new and specially designed for this work, the apparatus is essentially a duplicate of that employed with the albino rat, elsewhere described[40] and figured.[41] Though this closed-circuit apparatus has been used in the majority of our measurements, the reader is referred to the above-mentioned sources for the essential description of the apparatus. The special respiration chambers for dove and pigeon, and a few points concerning the construction and use of this apparatus, require description here.

The respiration chamber. The respiration chamber for one ring dove is a rectangular, nickel-plated, copper vessel with double walls and built in two parts—base and cover. The space between the walls of the base is filled with water, and the edge of the cover fits into the water trough of the low base. Though the sides of the cover are of metal the top is of glass, recessed. To insure tightness between the glass and the metallic part of the cover, the glass is well waxed around the edges. A 5-mm. layer of water may also be poured over this seal for further security against a possible leak. The internal dimensions of the chamber are $12 \times 25 \times 15$ cm. (width,

[40] Benedict, F. G., Abderhalden's Hand. d. biolog. Arbeitsmethoden, 1924, Abt. IV, Teil.10, Fig. 221, 538.

[41] Benedict, F. G., and Grace MacLeod, The heat production of the albino rat. I. *This Journal*, 1929, 1, 343.

length, depth, respectively); the external dimensions are 14.5×27.5×16 cm. The apparent volume of the inner chamber with the cover on is 4781 cc.

The further description of this chamber will be aided by reference to an essentially similar chamber developed for use in an open-circuit system (shown in Figure 1). In the bottom of the chamber are two main openings of 4 mm. brass pipe, the one at the right-hand side leading directly to the absorbing vessels, the one at the left-hand side communicating both with the pipe coming from the blower and with the spirometer. In actual practise, and in order to have the air current flow backward along the bird's body, we have reversed these two connections from the positions shown in the drawing; that is, the air is made to enter at the right end of the chamber where the head of the bird is, and where this end of the bird-cage is suspended. To aid in controlling the temperature of the respiration chamber the entire chamber is surrounded by a wooden casing lined with asbestos paper. A small cover in the top is removable when one wishes to inspect conditions inside the chamber through the glass in the chamber cover. Further temperature control is obtained by means of a small, simple, heating unit, *H*, of nichrome wire in series with an external rheostat. This heating unit is placed beneath the chamber. In more recent work a bi-metallic thermostat has been placed in the air space between the respiration chamber and the wooden housing, to hold the temperature inside of the chamber at the optimum point.

In experiments with common pigeons a somewhat larger chamber is necessary, but it is possible to utilize the same spirometer, the same absorbing vessels, and the same ventilating system, as were designed for small doves. Utilizing two sets of 3-way valves, and appropriate connections, either dove or pigeon chamber can be hooked up with the rest of the respiration apparatus. The pigeon chamber is built on the same principle as the dove chamber just described, save that the base is lower (5 cm. high), and the cover (representing the main part of the chamber) is of sufficient size to cover the bird cage and have the lower end dip well into the water seal in the base. The internal dimensions of the base are 14×35× 5cm. (width, length, depth, respectively); the external dimensions are 16×35× 5 cm. The top or cover has internal dimensions of 15×34.5×15.5 cm., the glass in the cover being recessed 2.5 cm.; the external depth of the cover is 18 cm.

Bird cage. The bird is placed inside of the chamber in a light-weight cage (*O*, in Figure 1) of perforated metal, with a hinged, sloping cover at one end of its top. The lighter (left) end of this cage rests on a small plat-

form having a knife-edge. The heavier end, toward which the head of the bird is directed, is partly suspended by means of a hook and a small spring (or a rubber band). Under this same end of the cage is a small rubber ball, whose volume is affected by each movement of the cage, and which communicates with a capillary copper tube (1 mm. hole) leading out through the bottom of the chamber to a tambour writing on a kymograph drum. This arrangement provides for the graphic registration of the degree of activity (or repose) of the bird during the period of observation. On the top of this cage is fastened a thermometer, so placed as to be visible through the glass cover of the chamber (after removal of the wooden cover to the small opening in the top of the wooden casing). This thermometer records the temperature of the respiration chamber. In recent work it has been found much better to blacken the glass cover and to insert the thermometer through it, thus permitting the temperature to be read without admitting light into the chamber. The bird within this chamber is always placed with its head directed toward the incoming air. Otherwise the slight air current moves the bird's feathers, and this degree of discomfort may cause the bird to try to turn around.

Ventilating system. The actual amount of air required to pass through this respiration chamber per minute is, for the dove or pigeon, very small. Theoretically, the carbon dioxide content of the air in the system is supposed to be held at not far from 1 per cent or below. To do this effectively, a ventilation of from 500-1000 cc. per minute is all that is necessary, even with common pigeons; and we have used the same rate for dove and pigeon.

It was found difficult to secure a small rotary blower which would, with sustained certainty, move such a small volume of air and at the same time be air-tight. It therefore became necessary to reconstruct one of the prevailing types of blowers in the market in the manner described below. The ordinary Cenco rotary blower is built upon the principle that at high shaft speed the blades in the rotor, which are of hard rubber and are weighted with lead, fly out by centrifugal force against the periphery of the rotor chamber, and thus force the air along. At the slow shaft speeds necessary for our slow rate of ventilation the rate of rotation is not sufficient to hold the blades against the wall of the rotor chamber. Hence small flat springs were placed behind each rotor blade. Reduction of the shaft speed to such a point that the rate of ventilation would not exceed 1 liter per minute was accomplished by attaching a small worm gear to a standard electric motor. This motor is mounted on the same table that supports the other parts of the apparatus, and a chain drops down from the motor and connects with the sprocket on the rotor shaft of the blower beneath the

table. The whole blower is immersed in automobile oil of light body to a depth of from 10 to 12 cm., and the pipes leading to and from the blower are connected with ground-joint metallic couplings below the oil to insure absence of leaks. By adjusting the speed of the motor, or by adjusting the size of the sprockets and the speed of the rotor, any desired rate of ventilation can be secured. Obviously the speed control is much easier with a direct current than with an alternating-current motor. In the former case line resistance is the simplest procedure.

Absorber cans. The air leaving the chamber contains carbon dioxide and water vapor, both of which are removed as the blower sucks the air through appropriate reagents. Calcium chloride is used to absorb the water vapor and soda-lime to absorb the carbon dioxide. These reagents are contained in ordinary one-pint varnish cans. The central cap at the top of the can is soldered on, and a copper thimble (fitted with a one-hole rubber stopper) is also soldered to one side to serve as an opening for filling and likewise to permit the exit of the outgoing air. The air enters the can through the central cap, passes down through the reagent and up through the tube in the copper thimble; the lower end of the tube has four openings in its walls, and these openings are protected with a cap of close-fitting wire gauze to prevent clogging by the reagent.

Two of these cans (here called *a* and *b*)*, each containing 240 grams of calcium chloride, suffice to remove the water vapor completely from the air current, and by weighing these separately one can control easily the efficiency of the first can and remove it as soon as the second can begins to gain in weight. These two calcium chloride cans, *a* and *b*, are for convenience placed below the table or platform on which the other absorbers rest. The dry air leaving these cans then ascends through a brass pipe leading to two cans (here called *c* and *d*) on the top of the table containing, respectively, soda-lime and calcium chloride. The soda-lime in the can, *c*, absorbs the carbon dioxide from the air and the calcium chloride in the can, *d*, removes the water vapor which is necessarily given off to the dry air by the moist soda-lime. Since the soda-lime is only slightly moist, the amount of water vapor carried over is small and the calcium chloride vessel, *d*, retains its efficiency during several experiments. But *c* is renewed as soon as the combined gain in weight of *c* and *d* has reached 10 grams. The calcium chloride can used in position *d* is never used in this position after it has gained 8 grams. This vessel, after an 8-gram gain, may still be used for some days in position *a*, below the table. Similarly the second

* The italic letters in this description do not refer to Figure 1.

calcium chloride can, *b*, is not used in that position after it has gained 8 grams. Calcium chloride, CP, anhydrous, 8 mesh, granulated, is used.

The several patented forms of soda-lime on the market are all too moist to be used with the dove or pigeon apparatus, since the water vapor given off by such soda-lime rapidly exhausts the calcium chloride following it. The modified Haldane soda-lime has been found most satisfactory.* Approximately 310 grams of this soda-lime are used in each can. This amount will absorb 10 grams of carbon dioxide before becoming inefficient (this applies to the total gain of the soda-lime and the can of calcium chloride which is hooked up with it).

Determination of Carbon Dioxide Produced

Theoretically, the air leaving the calcium chloride can, *d*, is at the same degree of humidity (i.e., thoroughly dry) as the air entering the soda-lime can, *c*. Hence the increase in weight of these two cans, *c* and *d*, represents the weight of carbon dioxide absorbed. The cans used by us are too heavy to be weighed on the ordinary analytical balance. On the other hand, the the ordinary U-tube, though weighable on this balance, requires such frequent changing (due to exhaustion of the reagent) that it has seemed best to use somewhat larger containers and to have the period of experimentation long enough to insure the collection of sufficient carbon dioxide, so that the latent error of weighing will be negligible. The only low-priced balance found which weighs these cans with a sufficient degree of sensitivity is the so-called "3-kilogram balance of Sauter."** This balance permits the weighing of one or both of these cans to within 5 mgs., and if not less than one gram of carbon dioxide is collected, the error thus becomes physiologically insignificant.

Spirometer and Determination of Oxygen Consumed

During the time that the bird is in the closed chamber, it is absorbing oxygen from the air. The volume of air in the apparatus is thus decreased, and if no regulatory mechanism were available, a distinctly negative pressure would develop. To avoid this, a spirometer is attached in direct communication with the chamber at the point where the air enters the chamber. Near this point is also a petcock through which oxygen may be introduced. The spirometer is of simple type, having a light-weight bell

* The method of preparing the modified Haldane soda-lime has been previously described by F. G. Benedict in Abderhalden's Hand. d. biolog. Arbeitsmethoden, 1924, Abt. IV, Teil 10, p. 449. It may be secured from W. E. Collins, 555 Huntington Avenue, Boston, Mass.

** Made by August Sauter, Ebingen, Württemberg, Germany. Balance No. 511 (old No. 8 II b).

delicately counterpoised at its middle point and having a pointer on the counterpoise traveling over a millimeter scale. This spirometer offers no resistance to the passage of air. As the volume of air in the system decreases, due to the combustion of oxygen in the tissues of the bird and to the absorption of the carbon dioxide produced, the bell descends and allows its gas to escape into the respiration chamber to compensate for the decrease in volume. The change in level of the bell may be read directly upon the scale attached to the spirometer, and this change in level is a measure of the apparent oxygen consumed.

The spirometer bell has a total volume of not far from 1 liter. It is 78 mm. in internal diameter, and each millimeter of vertical movement corresponds to an apparent volume of 4.87 cc. In the calculation of the oxygen consumed, reduced to O° C. (dry) and 760 mm., due allowances should be made for the temperature of the spirometer bell, the temperature of the chamber, and the average temperature of the absorbing vessels, as well as for the changes in barometric pressure. It has been found that changes in humidity throughout the system may be disregarded. The chamber is made hermetic with a water seal, the spirometer bell is immersed in water, not infrequently urine is voided, and the air inside the chamber, although admittedly not completely saturated, is assumed to have a constant water content.

This method of measuring the apparent oxygen consumption, by noting the change in level of the spirometer bell, has not been employed by us in actual practise. We have preferred to meter the oxygen by means of a simple pump of known volume and introduce the metered oxygen from this pump directly into the spirometer. For this purpose the experiment is begun with the spirometer bell at a fairly low point, a definite volume of oxygen is introduced from the pump through a petcock, the bell fills with oxygen (i.e., ascends to a higher level), and the experiment is continued until the bell has descended to the starting level. In this way the spirometer is used simply as a "zero" instrument, and changes in temperature and pressure have a minimum effect. When it is not convenient to continue the experiment until the spirometer bell has returned to its original level, the factor of 4.87 cc. (representing the apparent volume of each millimeter vertical movement of the bell), has been used to correct for the amount of oxygen introduced from the pump, but not actually used. Since the volume of the bell is about 1 liter, it is possible to introduce this amount of oxygen at one time, when experiments of long duration are desired. Ordinarily oxygen is introduced at the rate of 370 cc. at a time (i.e., the volume of the pump). From the known volume of oxygen introduced, and

the length of time required for the bell to reach its original level, the apparent volume of oxygen consumed per minute may be calculated, and reduction of the apparent volume to O° C. (dry) and 760 mm. is made in the usual manner.

The pump used in this work was developed at the Nutrition Laboratory; it has been used extensively in many of its researches, and has already been described in detail.[42] The pump has a constant length of stroke of 200 mm. and a capacity of approximately 370 cc. for each full stroke. To insure constancy in length of stroke, the handle comes in contact with the top of the pump before the piston or plunger reaches the bottom, so that the contact is metal to metal.

A rubber bag,[43] preferably that used for the interior of basket balls, is recommended as a holder of pure oxygen in these tests. The bag, with slight excess of water inside to insure saturation of the oxygen with moisture, can be filled with oxygen from a cylinder of the compressed gas. If there is any appreciable tension (more than 10 to 20 mm. of water) upon the oxygen bag, the pressure in the pump should be momentarily relieved before its contents are discharged into the apparatus.

Typical Calculation of the Results of an Experiment with the Closed-Circuit Respiration Chamber for a Single Dove

In a typical experiment with a ring dove (132 gm.) studied 24 hours after taking food, during a period of repose, the carbon dioxide production, the oxygen consumption, and the respiratory quotient are calculated as follows:

Weight of CO_2 absorber cans	End	88.170 gm*	
" " " " "	Start	87.166 "	
Total CO_2 produced by dove	Diff.	1.004 "	

		Log.
Weight of CO_2 absorbed	1.004	0.00173
Grams per liter CO_2	1.9652	0.29340

Volume CO_2 produced by dove $= -1.70833 = 0.5109$ liters CO_2

[42] Benedict, F. G., *Boston Med. and Surg. Jour.*, 1927, CXCVII, 1165. See also, Benedict and MacLeod, *This Journal*, 1929, I, 343.

[43] Benedict, F. G., *Boston Med. and Surg. Jour.*, 1927, CXCVII, 1165.

* These are not the true weights of the absorber cans, but represent the additional weights added to the balance pan after the counterpoise was put on.

	Time	Spirometer level	Cage Temp.	Barometer temp.	Barometer	Correction brass scale*	Corrected barometer
Start	11:40 A.M.	60 mm.	25.0°C.	24.0°C.	766.4 mm.	−3.0 mm.	763.4 mm.
End	4:59 A.M.	60 mm.	25.8°C.	24.3°C.	762.7 mm.	−3.0 mm.	759.7 mm.
	5 hr. 19 min.		25.4°C. avg.				

	Logarithms Start	End
Volume of respiration chamber (4781 c.c.)	3.67952	3.67952
Reduction to 0°C. (dry) and 760 mm.*	−1.94984	−1.94622
	3.62936 = 4259.5	3.62574 = 4224.2 c.c.

Change in volume of air in chamber due to temp. and pressure changes ..... 4259.5 − 4224.2 c.c. = 35.3 c.c. decrease

	Pump temp.	Barom. temp.	Barometer	Brass scale corr.*	Corr. barom.
1 pump O_2 at 11:41 A.M.	24.3°C	24.0°C.	766.4 mm.		
1 " " " 1:43 P.M.	24.2°C.	24.1°C.	765.1 "		
Avg.	24.3°C.	24.1°C.	765.7 "	−3.0 mm.	762.7 mm.

	Log.
Volume 2 pumpfuls O_2 (741.0 c.c.) =	2.86982
Reduction 0°C. (dry) and 760 mm.* (762.7 mm. at 24.3°C.) =	−1.95143
	2.82125 = 662.6 c.c. O_2
Correction for decrease in volume of chamber	35.3 " "
Total O_2 consumed by dove	697.9 " "

Respiratory quotient $\frac{CO_2}{O_2}$ $\frac{.5109 \text{ liter} = -1.70833}{.6979 \text{ liter} = -1.84379}$

$-1.86454 = 0.73$ R. Q.

Calculation of the carbon dioxide production and the oxygen consumption is made to the one-hour or four-hour basis by multiplication by 60 or 240, after division by the number of minutes in the period. The heat production is computed by multiplying the oxygen consumption by its known calorific value per liter (4.714 large calories in this particular case)[44] at the determined respiratory quotient. Since the adult ring dove weighs on the

* These factors and logarithms will be found in the book of tables published by T. M. Carpenter (*Carnegie Inst. Wash. Pub.* No. 303A, 1924), i.e., Table 4 for the brass scale correction for the barometer and Tables 7 and 8 for the reduction of volumes of saturated air to 0°C. (dry) and 760 mm. pressure.

[44] Carpenter, T. M., *Carnegie Inst. Wash. Pub.* No. 303A, 1924, Table 13, 104.

average about 150 grams and many adult common pigeons about 300 grams, for comparative purposes we have computed all of our results on the basis of the heat production (expressed in small calories) per 150 (dove) or per 300 (pigeon) grams of body weight per 4 hours.

Open-circuit Respiration Apparatus for a Single Pigeon

As a result of an extensive series of experiments with both the common pigeon and the ring dove it was found that under the conditions maintained by us, that is, with the birds fasting at least 24 hours, the respiratory quotient was usually about 0.72. It was therefore decided that the carbon dioxide production alone could be measured in many experiments, thus increasing the number of experiments without necessitating the use of the complicated closed-circuit and the oxygen determination. Hence an apparatus was constructed on the open-circuit principle, primarily for the purpose of determining only the carbon dioxide excretion. The chamber (*C*, Figure 1), which has been used mainly for common pigeons, is in general design the same as the chambers prepared for use on the closed circuit apparatus. It consists of a water-trough base and a removable, metallic cover with glass top. In this particular case the base is made 10 cm. high and, by suspending the cover on U- hooks at different levels in the water seal, one can accommodate any size of cage and at the same time reduce considerably the extraneous air inside the chamber. Thus we have used this type of chamber for both doves and pigeons. The cage rests upon a platform with the usual type of suspension and connection, *t*, with a kymograph for recording the activity. The chamber is provided with an asbestos-lined, wooden housing and with suitable heating arrangements (similar to those described for the closed-circuit apparatus), so that the temperature of the chamber can be held constant.

The ventilating system is entirely different from that used with the other apparatus. Since it is necessary to determine only the carbon dioxide production, provision is made to supply air free from carbon dioxide to the chamber and to withdraw the air from the chamber by means of a blower, which forces it through sulphuric acid to remove the water vapor and through soda-lime to remove the carbon dioxide. Outdoor air, drawn into the chamber, is previously freed from carbon dioxide by passing through a large bottle of soda-lime, *D*. Large tubes with hose couplings 19 mm. in internal diameter are used on this bottle in order that the air may have free access from outdoors into the bottle, and in order to produce a minimum suction inside the respiration chamber. The pipe leading the ingoing air to the base of the chamber is 9 mm. in internal diameter.

As the air leaves the chamber it contains carbon dioxide and water vapor. It passes first through an empty bottle or trap, *a*. After passing through this trap, the air goes to a Cenco blower, *B*, which is connected with a high-speed motor. This blower is provided with a by-pass to reduce the effectiveness of ventilation. Leaving the blower, the air enters a Williams bottle, *b*, containing sulphuric acid, where most of the water vapor is

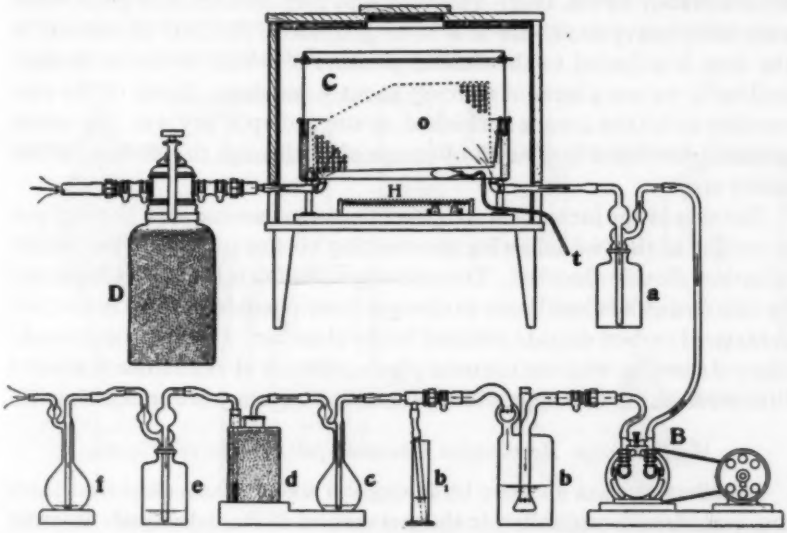

FIGURE 1. *Open-circuit respiration chamber for measuring the carbon-dioxide production of a single common pigeon.*

D, bottle of soda-lime to remove carbon dioxide from ingoing air; C, respiration chamber; o, perforated metal cage; t, tubing connecting rubber bulb beneath cage, o, with tambour outside chamber; H, heating unit; a, trap; B, blower; b, Williams bottle containing sulphuric acid for absorption of water vapor in outcoming air; b_1, safety trap serving as escape valve for release of excess pressure; c, flask containing sulphuric acid, serving as check on Williams bottle, b; d, can of soda-lime for removal of carbon dioxide in outcoming air; e, bottle containing sulphuric acid to remove water vapor given off to the air by the soda-lime in can, d; f, flask with sulphuric acid serving as check on bottle, e.

absorbed. It then enters a small glass flask, *c*, containing a small amount of sulphuric acid, which acts as a check on the Williams bottle, *b*. From there the dried air passes through a soda-lime can, *d*, where it is deprived of its carbon dioxide. The water given off to the air by the soda-lime is absorbed by sulphuric acid in the gas-washing bottle, *e*, and the glass flask, *f*, which acts as a check on the gas-washing bottle. From the flask, *f*, the air escapes into the room.

Considerable pressure is required to drive the air through this train of bottles, and in order to avoid any danger of the train blowing up, a trap, b_1, is provided (after the first Williams bottle,b) as an escape valve. To the metallic side of an ordinary hose coupling a short length of copper pipe (approximately 5 mm. outside diameter) is soldered. To this is connected a short length of rubber tubing, attached at the other end to a glass tube approximately 12 cm. long. This tube dips into mercury in a glass vessel with fairly heavy base, such as a 50-cc. graduate. The level of mercury in the trap is adjusted to the desired pressure at which release is wished; ordinarily we use a layer of mercury about 4 cm. deep. If any of the connections to bottles or cans are kinked, or stopped up in any way, the excess pressure developed is released by escape of air through the mercury in this safety trap.

The sum of the increase in weight of the soda-lime can and the increase in weight of the two following gas-washing bottles represents the weight of carbon dioxide absorbed. The rate of ventilation is kept fairly high, and by this means it is legitimate to disregard any possible changes in the percentage of carbon dioxide residual in the chamber. Under normal conditions of running, with one common pigeon, the rate of ventilation is about 1 liter per minute. This apparatus has been used in many of our experiments.

Multiple-cage Respiration Chamber for Groups of Pigeons

In collecting data on these birds singly it soon became clear that there was considerable variability in the metabolism of the individual. In order to secure a more general picture of the heat production of birds of the same race, sex, or type, we planned some measurements of *groups* of birds, usually from six to ten. The average value found with this group of birds would give a more general picture of the influence of a superimposed factor than would the values found with any one bird. To this end a respiration chamber (*D*, Figure 2) for determining the carbon dioxide only, based upon exactly the same principle as chamber C (described above), has been employed. The large water-sealed base is of sufficient size to hold a rack containing ten compartments or cages of perforated metal for ten common pigeons. Each of these compartments is $28.5 \times 11.4 \times 12.7$ cms. The dimensions of the block of ten cages, which is in two tiers of five each, are $28 \times 57 \times 25.5$ cms. The entire front of this block of ten cages is fitted with a thin, sliding door, made of perforated metal which permits the entrance, exit and enclosure of the birds. To prevent the dropping of urine through the cages, a small sheet of aluminum is placed in the bottom of each cage. The cover just fits over these cages, giving small clearance,

and has external dimensions of 30×60×27 cms. When the cover is dropped into the water seal the chamber is air-tight. By furnishing to the chamber carbon dioxide-free air (as in the case of chamber C), then withdrawing the air by a blower and absorbing the carbon dioxide and water vapor, it is possible to measure in each experimental period the carbon dioxide given off by ten pigeons instead of one. Furthermore, the amount of carbon dioxide produced will be such that the periods may be made much shorter and may be repeated as often as desired. We have uniformly measured four successive periods, each one hour in length.

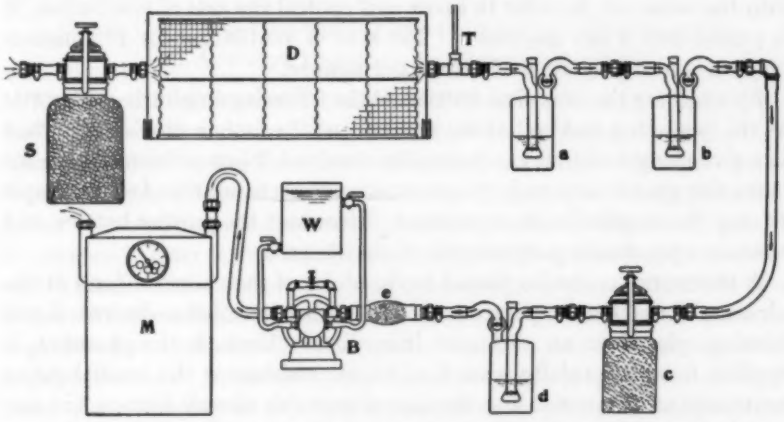

FIGURE 2. *Multiple-cage, open-circuit respiration chamber for measuring the carbon-dioxide production of groups of common pigeons.*

S, bottle of soda-lime to remove carbon dioxide from ingoing air; D, 10-cage respiration chamber; T, thermometer; a and b, Williams bottles of sulphuric acid to absorb water vapor from outcoming air; c, bottle of soda-lime to remove carbon dioxide from outcoming air; d, Williams bottle of sulphuric acid to absorb water vapor given up by the soda-lime in bottle c; e, tube containing sodium bicarbonate to remove acid fumes; B, blower; W, can of water for cooling blower; M, meter.

A soda-lime bottle, *S*, of standard size, but with large aperture, is provided to supply carbon dioxide-free air to the chamber as drawn in by natural draft. The pipe leading from this soda-lime bottle to the respiration chamber (i. e., at the intake point) is 1.8 cm. in internal diameter, thus preventing the danger of air being sucked into the chamber through the water seal. The blower, *B*, in the ventilating system is a Cenco blower, directly connected to the armature shaft of an electric motor. Since this apparatus requires a higher speed of ventilation and there is a possibility of the blower becoming over-heated, provision is made for cooling the

Cenco blower by suspending a vessel of water, *W*, above it and making two simple connections with rubber tubing to a water jacket which is an integral part of the blower. Thus a natural thermo-syphon system operates here and keeps the blower cool. By means of a by-pass the effective discharge of the blower can be readily controlled.

The purposes of the several parts of the system will be evident from Fig 2. The air finally enters the Cenco blower, first passing through a small tube containing sodium bicarbonate and cotton batting to remove any acid fumes and keep them out of the blower. The air is then discharged into the room; or, in order to know and control the *rate* of ventilation, it is passed into a dry gas meter. The rate of ventilation for 10 common pigeons is normally about 12 liters per minute.

By weighing the soda-lime bottle and the following sulphuric-acid bottle at the beginning and end of the experiment the carbon dioxide absorbed in a given length of time can be readily obtained. No provision is made for deflecting the air current from one set of purifiers to another, for it is simple to stop the ventilation for a moment, disconnect the purifier bottles, and connect a previously weighed pair of absorbers.

A thermometer can be placed in the cover of the chamber (and at the air-exit, *T*) and the temperature of the chamber recorded as desired. Local heating, placed in an enclosure immediately beneath the chamber, is applied from a specially wound nichrome resistance; the small heating units sold to be screwed into the base of portable electric heaters are also satisfactory. These heating units, used singly with 110 volt current, have 22 ohms and take 5 amperes. We have ordinarily used them in series, under which conditions they take 44 ohms and 2.5 amperes. With external resistance either in the form of lamps or so-called "*Dim-a-lites*," almost any degree of heating can be secured. The air intake should be located close to the top (not at the center as it is shown in Figure 2) of one end of this chamber in order to prevent the establishment of uneven and higher temperatures in the upper half of the chamber. We deflected this air current upward by soldering a solid strip of thin metal (at a distanc of 1 cm.) over the intake; this fan-shaped metal strip, fastened to the inside of the chamber cover, reached to within 4 cm. of the top. Thus all of the incoming air was really released at the top of the chamber.

Modification of Multiple-cage Respiration Chamber for Groups of Doves

Since the ring dove weighs only half as much as the common pigeon, and hence occupies much less space, it was felt that although ten doves could be placed in a small cage inside the chamber intended for ten common

pigeons, it would be, experimentally, a little better to reduce the volume of the chamber not occupied by the birds. A specially designed cover was therefore constructed to fit into the same base as that used for chamber D, but having an off-set so that it would both cover the ten cages in two tiers of five each and at the same time cut down the extraneous volume.

In chamber D and its modification there is no glass in the cover, and there is no provision for activity records. It was thought that the average activity of a group of ten birds would be essentially the same as that of another group of ten.

Glass Cage for Control of Environmental Temperature and Activity Previous to Metabolism Test

The seasonal temperature fluctuations of the laboratory and of the animal buildings were at first such that the birds were studied in the respiration chamber after having lived at considerably varying temperatures beforehand; and, though a standard chamber temperature during the metabolism experiments was insisted upon, the results usually could not be duplicated in later tests with the same animals. As a result of these tests, and of the Nutrition Laboratory's experience with albino rats at Columbia University, it was believed that with these birds the control of the environmental temperature for 24 hours prior to the respiration test would be highly desirable. In addition, the actual progress of the tests had indicated that still other factors than temperature should be carefully eliminated or leveled during this 24–hour preparatory period. A special cage or house was therefore devised, in which the birds might be kept for 24 hours prior to the respiration experiment at a known and constant environmental temperature, and in which certain activities of the birds could also be brought under control.

The cage devised is of sufficient size (2×1×1 meter) to permit fairly free flight of the birds, when flight is desired. It is rectangular in shape. Its sides and top are made of ordinary window sash, doubly glazed, with so-called "double-thick" window glass. The dead-air insulating space between the panes of glass is about 11 mm. thick. The front of the cage consists of a large door (also doubly-glazed) and a small permanent transom at the top. The interior of the cage is lined with chicken wire, so that the glass can not be broken by birds in flight. The extreme rear of the cage is provided with some very small, wire-sided compartments into which birds are first placed during the 2–hour period when they may regurgitate food, and also during the final 2–hour period preceding measurement, when all active flight is to be prevented. A false floor of

centimeter-mesh wire is also set slightly above the true floor so that food, if regurgitated at any time, can not be later obtained and eaten at a period less removed from the time of measurement. Sufficient ventilation, through holes in the top and bottom of the sash, was provided to insure that the carbon dioxide percentage inside the cage would never exceed 1 per cent. Finally, this large cage is divided into an upper and a lower half by a floor or partition of centimeter-mesh wire. Two birds (usually a mated pair) can be put together into each division. This has been found to be of real importance, since otherwise the jealous, fighting males will the more quickly exhaust their food stores and be measured (24 hours later) at a more advanced state of fasting than that of their mates.

During the greater part of the year the problem of controlling the temperature in this glass cage is one of heating. This is accomplished by a series of electric heaters, using special nichrome resistances and "units" employed in small electric heaters with screw base. These are placed near the floor in the rear of the cage. Bi-metallic thermostats were passed through the frame of the sash near the top and connected with each of two heaters. By means of a three-way snap-switch the control could be adjusted as desired. Since each thermostat would carry only about 1–1/4 amperes it was necessary to use two of them.

It has also proved necessary, however, to provide for cooling this cage during occasional periods of highest summer heat. To accomplish this, 22 meters of thin coiled copper pipe were placed along the sides and top of the glass house; through these coils cold water is passed in variable quantity whenever it is required. Since means of heating and of cooling are both provided, the temperature of this cage can be held at all times of the year at the point desired.

Concluding Statement

An extensive program of investigation of the basal metabolism of doves and pigeons has made it necessary to devise apparatus and technique especially suitable for use with these and similar small animals. In this work the technical requirements are rather severe because the ultimate purpose of our study is concerned with such small differences as may exist between the sexes, between different races or strains, and between various hybrids and their parent races.

The special sources of interest in the basal metabolism of these animals are noted, and the literature concerning metabolism measurements on doves and pigeons is briefly cited.

The various types of apparatus devised and used in these studies are

described here or elsewhere in such detail as will permit their duplication wherever wanted. These types include: A closed-circuit respiration apparatus for a single dove, and another for a pigeon; an open-circuit apparatus for a single pigeon; an open-circuit multiple-cage respiration chamber for 10 common pigeons, and a modification of this for use with 10 doves.

For most purposes it is necessary to use an apparatus with an activity-recording device. With such an apparatus three or four independent measurements should be made in successive "periods," (in closed-circuit apparatus the time used in the absorption of one pumpful of oxygen, about 370 cc., supplies a definite period), and the kymograph records then used to eliminate from consideration those periods not measured in repose. The multiple, or 10-bird apparatus, which is without an activity-recording device, uniformly gives values that are too high in tests with common pigeons, because activity can not be thus eliminated.

Details are given for the construction of a large, special glass cage in which the birds remain at constant temperature during the 24 hours preceding their measurement. This cage, and the arrangement of birds within it, must permit an adjustment of both the environmental temperature and the activity of the birds during this period; the quarrelsome, fighting males can thus be restrained from an early or unequal exhaustion of their energy stores.

Various accessories and reagents suitable for use with the different types of apparatus have been thoroughly tested and described. The data establishing the suitability of these appliances, in testing the various physiological factors affecting the metabolism of pigeons (Part II), are supplied in the communication immediately following.

THE MEASUREMENT OF THE BASAL HEAT PRODUCTION OF PIGEONS

II. PHYSIOLOGICAL TECHNIQUE

By

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The Birds: Their Nature, Variety and History

DOVES and pigeons are classified under two avian families—the Peristeridae (ring doves), and Columbidae (common pigeons). The ring dove and the common pigeon differ more than the rat and the mouse; their accepted classification accords them a difference equal to that between the rat and the beaver. It should therefore be distinctly understood that two very different animals are being investigated in the present study.

Races of Common Pigeons

Not one, but 13 races of common pigeons are under investigation. Three long-established races—Runts, Tipplers, Magpies—require a special statement. The Runts are perhaps the largest race of domesticated pigeon now in existence. Their weight averages about 550 grams. The Tipplers are almost the smallest of the 200 (domesticated) races of common pigeons, their weight averaging about 290 grams. The Magpies are of intermediate size. They are angular birds, with body, neck and legs notably elongated. Another very definitely charac-

Figure 1. *Feather from tail of "scraggly" common pigeon (left) and corresponding feather from a normal common pigeon (right).*

The "scraggly" feathers have little power to conserve body heat; some of them fail to develop at all, thus leaving small body areas quite naked.

[1] In carrying out this study we have had the technical assistance of Miss Edith Banta, to whom we are indebted for very faithful cooperation.

terized race, we call "*Scraggly*." The birds of this race have very defective plumage—every feather lacks those parts which conserve heat and provide support in flight (see Figure 1). Scragglies are therefore unable to fly, though they frequently attempt flight and though their muscular efforts are quite normal. Since their defective feathering and partly naked condition make them unable to conserve their body heat in the manner usual among pigeons, they offer a special opportunity for a study of the effect of rapid loss of heat on the rapidity of heat-production. A fifth race which, like the Scragglies just described, has been developed at the Station for Experimental Evolution, is called "Ataxic." These birds are unable to control their voluntary movements, and the neural basis of this disorder has been proved to be quite the same as that found in cases of human hereditary ataxia. Birds of this race fly occasionally, though backward nearly as often as forward. They can stand or sit still only by supporting themselves against some solid object. This race, like the Scragglies, is a special case of hereditary abnormality or disease. Consequently, results from these two races are ordinarily kept quite separate from results obtained with all other races of common pigeons.

Eight additional races of common pigeons have been studied, or are now being studied. During the last 8 years these races have been derived chiefly from a stock of common pigeons which was similar to the common laboratory pigeons now available in most parts of the world. These groups are perhaps more properly described as "strains" than as races, since pronounced or constant external characteristics do not attach to them. They are, however, lines inbred for at least 5-7 generations, during which period an attempt has been made to segregate races with large size, or with small size, of various endocrine organs. The measure of success attained in this particular effort with common pigeons is still somewhat uncertain. Certainly a good foundation of notable distinctions between these strains is provided by the circumstances that some of them carried the recently introduced blood of a pure wild species (*Columba livia*), and others carried either Homer or Jacobin blood.

Each one of this total of 13 pigeon races can be crossed with all of the others and a particular kind of hybrid thus secured. Obviously, these numerous kinds of hybrids can not be described here, though collectively they form a very important part of the material that is under investigation. Complete pedigrees make it possible to test whether, in any given kind of hybrid, the heat production is higher in the first (F_1) generation than in later generations. It is in this F_1 generation that the well-recognized "increased vigor" of the hybrid asserts itself, and also here,

in hybrids obtained from two very unlike species, that the sex ratio is much modified. A study of the heat production of this kind of hybrid, compared with that of the two parent races, provides an opportunity to learn whether the metabolism is at all related to the resulting hybrid vigor and to the modified sex ratio.

Races of Ring Doves

A total of 24 races or strains of doves is being studied. The blond ring dove (*Streptopelia risoria*), now commonly kept in cages throughout the world, contributed most to the original foundation of all these strains. Any of three other species, however, enters into the composition of some of them. One such species is the white ring dove (*St. alba*), which by some authorities is not specifically separated from the blond ring. Another is the Japanese ring dove (*St. douraca*); it is slightly represented in 5 of these races. Finally, the oriental turtle dove (*Turtur orientalis*), to the extent of one-sixteenth to one two-hundred-fifty-sixth of the total ancestry, is found in 10 races. Thus the original stock, from which many of our 24 races of doves were derived, was made up of species-hybrids. The order COLUMBAE is a most remarkable one in that the different species of the same genus usually show full fertility, one with another, and the resulting hybrids are also fertile.

The hybrid origin of several of these races has doubtless assisted the establishment of some special and important difference among them. During the last 6-7 generations a continuous and successful effort has been made to establish some races characterized by large thyroid glands, and other races by small thyroid glands. The choice of the particular pair of birds with which to found the race or strain was sometimes the most important element in this effort; and this choice was often made on the basis of the type of record for reproduction that had been shown by a pair of birds. We can now confidently predict that nearly every individual we may select from any of four races will have a thyroid of unusually large size; individuals from any of three or four other races will practically always have thyroids of small size. For a complete account of this matter the original publication may be consulted.[1] What these large or small thyroids mean in terms of high or low thyroid activity is, of course, a matter that will be decided by the present and future studies on the basal metabolism of these races.

Each of these 24 races may be crossed with all of the others and numer-

[1] Riddle, O., (In press, *Amer. Nat.*, 1929).

ous kinds of hybrids obtained. The statements previously made concerning hybrids between common pigeon races all apply here as well as there. In addition, the few well-defined "thyroid races" of ring doves provide an unrivaled opportunity to examine rates of heat production in individuals hereditarily provided with large, and with small, thyroids. Unfortunately, some of the races with extremes of thyroid size have become difficult to breed, and only a restricted supply of individuals is available for study during any single year. It is further probable that we have established two races (72 and 63a) of doves with small hypophyses, and two other races (36 and N2) with large hypophyses. It is certain that races 72 and 63a are characterized by notably shorter intestines than are found in races 36 and N2 (Riddle, unpublished data). If, as seems highly probable, intestinal length essentially reflects pituitary size, we have four "pituitary races" on which the gaseous metabolism is being measured. Also, we have made the various hybrid combinations of large and small pituitaries with large and small thyroids, and the heat production of these hybrids is now being studied. Probably no other material exists in which uncomplicated studies on basal metabolism can be made on these unique endocrine combinations.

Metabolic distinctions between these numerous races and hybrids are scarcely considered in this communication; but the details given above will make it clear that only birds of the same race can be grouped together in the data tabulated here. Also, this specially inviting diversity of our material—and the exact knowledge of the basis of this diversity—will further supply the reason for the present unusual effort to learn how to accomplish the accurate measurement of the basal metabolism of doves and pigeons.

General Statement

Since an examination of possible differences in the basal metabolism of the sexes is one of the principal ultimate objectives of our investigation, it seems necessary to note some of the physical conditions attending the rearing and management of these birds. Common pigeons of both sexes are kept until sexually mature (5-10 months old) in a large indoor-outdoor flying cage ($8\times5\times3$ meters); ring doves are kept in similar cages ($5\times1.6\times2$ meters), though these birds are not at all exposed to direct sunlight, nor to real winter temperatures, from December to March. As soon as birds are sexually mature a male and a female are given a separate cage ($0.6\times1.5\times2.2$ meters). In all periods of life the males and females are given identical space, sunlight and food. About one-sixth of the cages

for adult birds are provided with little or no direct sunlight, and special tests are being made on certain birds that have long remained in these cages in an effort to learn whether their metabolism has been affected by this condition. All of these birds are protected from extreme winter temperatures not only by glass walls to their quarters, but by heat artificially supplied.

Though the unqualified names—dove and pigeon—are considerably confused among English-speaking peoples we shall use the term "dove" to cover all of the 24 ring dove (mainly *St. risoria*) races; the word "pigeon" will apply to all (except Scragglies and Ataxics) of the 13 domesticated races of *Columba domestica* used by us. Accurate pedigrees extending over the last 15-25 years are available for nearly all of these races. During all months of the year these birds feed on a uniform mixed grain diet. One grain mixture, however, is suppplied to doves and another mixture to pigeons.

The quality of the living material for this study is of first importance, but its quantity has a practical significance. The few birds taken each day for measurement can be selected from rather more than 1000 individuals; and about 1000 young are reared each year. The exact age of every individual is known. The entire reproductive history, including the exact age of attaining sexual maturity, is known for every adult female. Soon after the gaseous metabolism is measured the birds, if adult, are killed and careful autopsies performed. Diseased individuals are thus sometimes found, and the measurements made on them are classified apart from results obtained on healthy birds. Only data from healthy individuals are utilized in this report. During 17 years the birds of this colony have been subjected to close and continuous study; very much of what has been learned about them has assisted our efforts to obtain trustworthy and classifiable measurements of their basal metabolism.

The General Preparation of the Birds for Measurement of Metabolism

In addition to a fasting period, a topic discussed at length in the next section, a number of other conditions, concerned with the preliminary treatment of the birds, have been investigated and found capable of affecting the rate of heat production. Some of these factors will now be considered, and the data concerning them presented. It should be stated at once that we have found it necessary to use the 24 hours preceding our measurements not merely as a fasting period, but as a period of

general preparation of the birds. During this 24-hour period the birds are kept at a uniform temperature and their activity is largely controlled in a large, specially constructed glass cage, a description of which has been given in the preceding paper (p. 493 of this number).

Amount of Food in Crop at Beginning of Fasting Period

Though food may be made constantly available to doves and pigeons this does not, as a rule, result in continuously filled "crops." The adult birds of our colony usually take food rather sparingly in the early part of the day (7:00-10:00 A. M.), and more freely during later daylight hours. These birds always feed by day, not by night; otherwise most rules of feeding are rather readily broken. It may therefore happen that at the time a bird is isolated for its 24-hour fast its crop may be empty

TABLE I

EFFECT OF FOOD IN THE CROP

Summary showing that some ring doves, among groups not hand-fed at the beginning of the 24-hour fasting period, give lower heat production values than when all begin this period with 5 grams of food in crop (day-runs at 30°; fasting cage at 30°).

Race	Birds not hand-fed at beginning of fast		Birds hand-fed when less than 5 grams in crop	
	Number birds tested	Average calories*	Number birds tested	Average calories*
63	9	3349	9	3744
62, N2	15	3505	17	3675

* Small calories per 4 hours per 150 gram bird.

or it may be full. It was found that when we used no means of assuring the presence of food in the crop at the beginning of the 24-hour fasting period we obtained, at the end of that period, lower heat production values than when the fast was started with food in the crops of all birds. Some data on this point are given in Table I. It therefore became necessary, at the beginning of the fasting period, to feed all birds by hand in the way described immediately below.

The assistant was trained to estimate accurately the amount of food present in the crop of a living bird. To get this training she estimated the amount (in grams) of food present in the crop of each of a group of birds that could be killed immediately; the estimates were then checked by killing the birds and weighing the food actually present. Only after the estimates became accurate was the task of "stuffing" the birds begun.

All ring doves have the amount of food in their crops brought up to 5 grams; for common pigeons this amount is 10 grams. It sometimes happens that these quantities are already present and that no feeding by hand is necessary. Water (by pipette) must also be given when birds are hand-fed; otherwise digestion is abnormally delayed.*

It is necessary to know that no bird regurgitates all or part of this food, and to re-feed in cases where regurgitation does occur. This is accomplished by placing each freshly fed bird for two hours in a small compartment within the constant-temperature glass cage (where it is to remain during the 24-hour preparatory period). Experience proves that the birds almost never regurgitate (Scragglies excepted) after this 2-hour period. But provision is made against the contingency that a bird may still later (at night) throw up food, and again eat this food next day when nearer to the hour at which its metabolism is to be measured. This provision consists in supplying the glass cage with a false floor of wire screening, through which such food must fall beyond the bird's reach. Water is supplied these birds during all except the final 5-6 hours of the preparatory period; its absence during these final hours is to make sure that no unabsorbed water is included in the bird's weight at the end of the measurement.

Finally, it is not a matter of indifference whether the fasting period begins (with 5 or 10 grams in crop) in the morning or in the evening. The course of the respiratory quotient, and a comparable stage in the fasting level, can be better predicted or identified when the fast is begun in late afternoon or evening (see Tables V and VI). This is to say that 5 or 10 grams of food, superimposed upon some preceding hours of feeding, (not upon a night of fasting), best prepare these birds for measurement 24 hours later. Comparable measurements can be obtained on these birds only by eliminating differences in the state or level of fasting.

Exercise and Flight During the 24-Hour Preparatory Period

It is obviously desirable that the birds should remain moderately quiet during the period immediately preceding measurement. It also appears that 24 hours of quiet, enforced by confinement in a very small space, would be undesirable; possibly it would modify the results, since, as noted later in this report, 35 days of such confinement very notably reduce the basal metabolism. These two points have been resolved as follows: Within the large glass constant-temperature cage, where the birds remain

* This fact was independently found, and first reported for both fowl and pigeon, by K. Ihnen (*Arch. f. d. ges. Physiol.*, 1928, CCXVIII, 783).

during this 24-hour period, a few small compartments (28×28×18 cm.) were built. Into these very small separate cages the birds are placed during the first 2 hours (to identify those which may regurgitate food). During the next 19 hours, however, the birds have approximately the amount of flying space to which they are accustomed. Two hours before they are to be placed in the respiration chamber they are again placed in the very small compartments where no flight and but little movement is possible.

In later and present work, in which only 4 birds are in this cage at the same time, this glass cage has been sub-divided by a wire screen partition into an upper and a lower half. A mated pair of doves or pigeons is placed together in one of these halves. This assures that the male of the pair does not rapidly and continuously use his energy reserves in fighting and driving a strange bird away from his mate.

Near the end of the 24-hour preparatory period the birds are transferred from these small compartments to small perforated metal cages in which they remain during the entire metabolism test. Meanwhile these perforated cages have been placed in, and have acquired the temperature of, the large glass cage; these perforated cages, enclosing the birds, are wrapped in heavy towels and transferred, with little change in temperature, to the previously heated chambers (20° or 30° C.) of the respiration apparatus.

Measurements Begin One Hour After Placing Bird in Metabolism Chamber

Our experience has shown that actual measurements should not begin until about one hour after the birds are placed in the metabolism chamber. As a rule, the birds are less quiet during this first hour than in succeeding periods, and there is also some activity on the part of the birds incident to their transfer to the metabolism chamber. With one type of apparatus used by us we have uniformly obtained a measurement of the metabolism during this first hour, though this has been rejected and our calculations based upon the measurements made during the next 3 hours only. In Table II data are given which permit a comparison of values obtained in this first hour with those obtained in the second hour. The first-hour values are higher by 3.4 per cent in ring doves, and by 3.3 per cent in common pigeons. This difference we assign to an increased activity and excitement during this first hour, or as an after effect incident to the transfer of the birds to the metabolism chamber.

In a later section(The Temperature Factor, etc.) it is shown to be

necessary to keep all birds at the same temperature in a specially constructed cage during this 24-hour preliminary period, if comparable heat production values are to be obtained from birds measured at all seasons of the year.

TABLE II

SHOWING HIGHER VALUES (THE RESULT OF ACTIVITY) DURING THE FIRST THAN IN THE SECOND HOUR

Birds are in the metabolism chamber (day-runs, at 30°, in 10-bird apparatus).

Test	Calories* from first (above) and second (below) hours	Test	Calories* from first (above) and second (below) hours	Test	Calories* from first (above) and second (below) hours	Test	Calories* from first (above) and second (below) hours
	Ring doves				Common pigeons		
1	4055 3782	6	2951 3033	1	7438 7100	6	7165 7393
2	4007 3511	7	3229 3335	2	6994 6337	7	7181 7028
3	3620 4006	8	3261 3036	3	5547 6012	8	7837 7563
4	4503 3469	9	3979 3923	4	8174 7091	9	7327 7584
5	3469 3793	10	3835 3780	5	6866 6641	10	6990 6444
Average (10) first hours = 3691 Average (10) second hours = 3567							=7152 =6919

* Small calories per 4 hours per 150 grams body weight (doves) or per 300 grams body weight (pigeons).

By way of summary it can be said that our results indicate that during the 24 hours preceding measurement, the proper general preparation of doves and pigeons for metabolism measurements involves the following items: 1.—A uniform fasting period (discussed later), best started in late afternoon or early evening. 2.—The beginning of this fast with uniform amounts of food in the crop, delivered by hand to the extent necessary (and accompanied by water). 3.—Means of knowing that this food is not regurgitated, and definite knowledge that no regurgitated food is available to the bird at a later part of the fasting period. 4.—A free supply of water until 5-6 hours before measurement. 5.—Means of preventing vigorous activity during the final two hours of the fasting period, but permitting usual activity during most of the fasting period. 6.—Preventing the males

of mated pairs from rapidly using their energy in fighting and driving. 7.—The maintenance of the fasting cage at a temperature uniform for all birds at all seasons (discussed elsewhere). 8.—A delay of one hour, after birds are placed in the metabolism chamber, before actual measurement begins.

The Respiratory Quotient and the Stage of Fasting Favorable for Metabolism Measurements

General Considerations

In basal metabolism determinations with animals it has been the custom to have the test preceded by a fast of 24 hours in order to secure absence of digestive activity. Based in large part upon our experience with wild birds,[1] and in some part upon the experience which other investigators have had with birds, we temporarily assumed in these studies with pigeons that a 24-hour period without food is prerequisite for securing standard and comparable conditions of measurement. This time interval was designed to rule out the immediate effects of strictly digestive activity and yet to have the metabolism experiments precede the period in which undernutrition or fasting has a marked influence.

This general procedure, carried out under standard conditions, is quite satisfactory for a comparison of various birds of the same species. But if the metabolism of these birds is to be compared with that of other birds or with that of mammals, especially man, it may still be questioned whether measurements made on doves and pigeons 24–30 hours after food correspond to commonly accepted basal conditions. Is it physiologically sound to compare the metabolism of pigeons measured 24 hours after food with that of man measured 12 hours after food? With humans there is a striking difference between the fasting and the post-absorptive condition. In the post-absorptive condition, which occurs with man 12 hours after food (if the diet has been a mixed one and not too rich in protein or calories), the respiratory quotient is in the vicinity of 0.82. With pigeons the respiratory quotient 24-30 hours after food has been found to be close to 0.72. This finding, insofar as the level of the respiratory quotient alone is concerned, would indicate that the pigeon's metabolism has reached a stage corresponding to the stage reached by man on the third or fourth day of fasting. Indeed, it has been argued that with small animals, such as these birds, the supposedly intense metabolism may make such heavy drafts upon body material that fasting for 24 hours

[1] Benedict, F. G. and E. L. Fox, Bicentenary Number, *Proc. Amer. Philos. Soc.*, 1927, LXVI, 511.

may really correspond to a fast of several days with larger animals. Since prolonged fasting lowers the metabolism profoundly, it is conceivable that the metabolism of these birds 24 hours after food is not "basal," but is a prolonged fasting metabolism.

Two problems thus present themselves. The bird's food is in large part carbohydrate in nature and the respiratory quotient should be high during the absorption of food. The first problem was therefore to note the changes in the course of the respiratory quotient during a prolonged period following the ingestion of food. The second problem, closely connected with the first, was to note the course of the total metabolism during hours and days following the ingestion of food.

To secure information on these points we made two series of measurements of respiratory quotients, and of metabolism, at known times after the taking of food. The measurements which will be described as a "summer" series were made at the Nutrition Laboratory where the use of delicate gas-analysis apparatus made possible the rapid determination of the respiratory quotient. In these experiments not only were determinations of the respiratory quotient made from hour to hour, or from half-hour to half-hour, but carbon dioxide measurements were made from which the heat production was calculated. This work was done with special apparatus which will not be described here, and with samples of the air leaving the darkened chamber analyzed on the Carpenter gas-analysis apparatus. The "winter" series of measurements was made at the Station for Experimental Evolution where the birds could be prepared and maintained under truly standard conditions and where the heat production could be more satisfactorily measured.

In such a study two items concerning the food habits and the digestive activity of these birds are of much importance. First, these animals voluntarily take food during daylight hours only. When 5 or 10 grams of food are administered to a bird in the early evening these amounts are added to other food rather recently consumed; but when these amounts are given in the early morning (particularly following the long nights of winter) they enter a food-free digestive canal. Second, the digestion of food occurs much more slowly in these birds than in man. The total time necessary for digestion and absorption is not yet definitely known, but the usual minimum time required for the food to be softened and passed from the crop to the gizzard has been found to be from 6–15 hours. Certainly additional time is required for the completion of digestion in the gizzard and intestine. It may therefore sometimes happen that the respira-

tory quotient, and the metabolism, will be first affected only some hours after food enters the bird's crop.

Time Relations in The Respiratory Quotient after Withholding Food

Though the respiratory quotient is commonly assumed to indicate the nature or character of the material burned, Jahn[3], in reporting observations made in 1928, contends that the respiratory quotient is not an index of the nature of the oxidative processes taking place in the tissues. For purposes of comparison and discussion, however, we may hold to the original assumption.

The data from the "summer" series will be described first. The respiratory quotients obtained from ring doves are given in Table III, and those for the common pigeons in Table IV in the chronological order in which the determinations were made. Examination of the data in these tables shows that in general the high quotients are in the upper portion of the table, indicating that the quotients approximating 1.00 more often occur shortly after the withdrawal of food. These suggest a combustion predominantly of carbohydrate. Of the quotients secured from the twentieth hour on, very few are high. By the twenty-fourth hour satisfactorily low (or too low) quotients were almost always found in this series of tests. In several instances the fasting level was reached between the sixteenth and eighteenth hours (some of the cases of low quotients at 6–10 hours may be instances in which little or none of the food last eaten had as yet been absorbed). On the other hand, there appears to be no regularity between the quotients and the time after feeding, earlier than 20–24 hours after food. A sudden drop in the quotients is observable in several cases. This matter is worthy of further study in its relation to the emptying of the crop and to the various stages of the passage of food along the intestinal tract.

Concerning these "summer" tests it should be further stated that the amount of water present with the food last given was often probably below the amount necessary to secure an optimum rate of digestion. This point, and a sharp differentiation between fasting periods begun in morning and evening, is fully covered in the data of the "winter" series which will next be described.

In the series of "winter" tests conducted at the Station for Experimental Evolution little attention was given to the first 6 or 8 hours after taking a bird and feeding it its quota of 5 or 10 grams of grain Obviously it

[3] Jahn, D., *Deutsch. Arch. f. klin. Med.*, 1928, CLIX, 335.

TABLE III

RESPIRATORY QUOTIENTS OF RING DOVES REFERRED TO HOURS AFTER FOOD ("SUMMER" SERIES)

	D305		D4	H132					D866				Bob			D880
Hours after food	May 10 11	May 12	May 17 18	Sept. 13 19	Sept. 14 15	Sept. 21	Sept. 24	Oct. 10 20	Sept. 18 17	Sept. 26	Oct. 18 10	Oct. 17 19	Oct. 1 Sept. 27	Oct. 4 Sept. 28	Oct. 9 (20)	Oct. 18 9 19
0–2			.94	.81							1.01		.96			
2–4	1.01		.98	.83							1.02		.94	.93		1.01
4–6	.94		.97	.83									.97	.96		
6–8	.81		.95	.83	.76								.96	.89		
8–10	.79			.78	.72								.82	.81		—
10–12		.90						.82	1.00		—		.80	.71	.95	.77
12–14		.78						.94	1.03	.92	.92		.76	.74	.97	.77
14–16	—	.78	—	—	—			.76	.91	.87	.93		—	—		.78
16–18	.73	.75	.73	.71	.74	.78	.72	.74	1.02	.75	.73		.69	.72		
18–20				.74	.72	.79	.74	—		.71	.74			.72	—	—
20–22				.79	.74	.77	.72	.74				.71	.71	.73	.76*	.72
22–24				.72		.74			—			.71	.65	.71		.70
24–26									.69			—				—
26–28									.69			.71				.71
28–30									.68							
30–32																.71

* Determination on dove H92 on October 20.

TABLE IV

RESPIRATORY QUOTIENTS OF COMMON PIGEONS REFERRED TO HOURS AFTER FOOD ("SUMMER" SERIES)

Hours after food	X843		X600	X664								R1	R2	
	May 7	May 15 16	Sept. 11	Sept. 13 19	Sept. 14 15	Sept. 21	Sept. 24	Sept. 25	Oct. 1	Oct. 4	Oct. 9	Sept. 18 17	Sept. 26 20	Oct. 10
0–2			.97	.87					1.07					
2–4	.92		.98	.93					1.06					
4–6	.80		.92	.95					1.03	.97				
6–8	.75		.85	.90	.75				.90	.95				
8–10	.72	.73		.79	.74				.78	.82				
10–12		.71							.77	.75	.72	.95		
12–14								.81	.77		.73	.92	.75	.88
14–16					—			.74	.74		.69	.88	.73	.74
16–18				—	.75	.75	.74	.73				.79		.72
18–20		—		.97	.77	.75	.76	.76						
20–22		.77		.86	.75	.74	.75						—	
22–24		.71		.77		.72	.73					—	.76	
24–26				.76								.70	.73	
26–28												.67	.73	
28–30												.72	.71	

may happen that at the moment a bird is thus caught and fed it is already in a near-fasting state with a low respiratory quotient and with a low metabolism. The difficulty is that one does not *know* its condition, and that *most* individuals thus taken would not be fasting but in various stages of alimentation. Again, since the food most recently given requires several hours for absorption, the period of chief interest is that following the tenth to twelfth hour after the last food. Finally, the *night* is the right time to measure the metabolism of these birds and measurements made from the twelfth to the twenty-seventh hour consume all, or more than all, of the night even when the time chosen for beginning the fast permits the beginning of measurement in the early evening. These considerations were uppermost in the "winter" tests, made during January and February.

Some data on the course of the respiratory quotients from the eighth to the twenty-ninth hour of fasting are given in Tables V (doves) and VI

TABLE V

RESPIRATORY QUOTIENT AND METABOLISM OF RING DOVES REFERRED TO HOURS AFTER FOOD. Measurements made in the usual manner (not by gas analysis), and at 30°— except as noted ("winter" series).

Hours after food	H325		H334	D804		H501
	R.Q.	Calories	Calories	R.Q.	Calories	Calories
Fasting started in evening						
12–16	.97	3521 I+	3319 I+	.96	3428 I+	3086 I+
16–21	.84	3229 I+	3289 II−	.80	3015 I+	3236 I+
21–25	.78	2644 I+	2522 I+	.75	2716 I	2314 I
25–27	—	2637 I+	2547 I+	—	2712 I	2320 I
Fasting started in morning						
	D871		D775	D705		D736
9–13	.77	3078 I+	3618 I	.74	2937 I	2995 I+
13–17	.84	2947 I+	3381 I	.84	2781 I	2950 I+
24–29*	.77	2691 I+	2862 I			
Fasting started in morning (run at 20°)						
	H895		C18	H942		H871
12–15	.74	5270 I+	4473 I	.71	5232 I+	4491 I+
15–17	.73	5403 I+	4673 I	.72	4959 I+	4351 I+
17–20	.76	6169 II−	4738 I	.73	5188 I	4076 I
20–23	.73	5259 I+	4797 I	.79	5672 I+	4247 I
23–26	.74	4208 I	4748 I+	.68	5616 I+	4486 I+

* This fast started in evening of another day.

Note. Roman numerals refer to activity (see p. 514).

(pigeons). In birds whose fast was begun in the evening, the respiratory quotients were already high at the eighth or twelfth hour of fasting (and the crops of all these 8 birds, except H501, still contained food at this time). These quotients all remained high to the nineteenth or twenty-first hour. That quotients from birds thus fasted may be expected to be low at 24 to 30 hours has otherwise been clearly shown. Among 100 such quotients consecutively obtained on ring doves values were distri-

TABLE VI

RESPIRATORY QUOTIENT AND METABOLISM OF COMMON PIGEONS REFERRED TO HOURS AFTER FOOD

Measurements made in usual manner (not by gas analysis), and at 30°— except as noted ("winter" series).

Hours after food	J42 (Scraggly)		X969	X345		X715 (Scraggly)
	R.Q.	Calories	Calories	R.Q.	Calories	Calories
Fasting started in evening						
8–10	.84	5443 I	5106 I+	.94	4108 I+	6710 II−
10–12	.82	6212 I+	4908 I	.96	4289 I+	6819 II−
12–14	.83	6422 I+	4929 I+	.94	4656 II−	6870 II−
14–16	.87	6896 I+	4951 I+	.86	5378 II−	7324 I+
16–17	.86	7168 I+	5525 II+	—	4880 II−	7488 II−
17–19	.82	7220 II−	4793 I+	.86	4968 II−	7664 I+
24–27	.74	6478 I+	4964 I+	.71	5279 I+	7147* I
Fasting started in morning						
	X592		X931	X917		X874
10–13	1.00	4363 I+	5179 I	.94	4368 I+	5097 I+
13–16	1.01	4526 I+	5225 I	.89	4443 I+	5010 II−
Fasting started in morning (all Tipplers run at 20°)						
		X695	M336		J143	M346
12–15	.75	6687 I+	6068 I+	.75	6565 I+	7237 I
15–17	.75	6653 I+	6500 I+	.75	6325 I+	7217 I
17–20	.81	6390 I	6093 II−	.79	6500 II−	6917 I
20–22	.82	6751 II−	6259 II−	—	6957 II	7590 I
22–24	.84	6586 II−	6405 I+	.79	7174 II+	7536 I+
24–26	.76	6752 II−	6487 I+	.77	6752 II+	7471 I+

* A respiratory quotient of .73 was found here.

Note. Roman numerals refer to activity (see p. 514).

buted as follows: 0.69 (5), 0.70 (9), 0.71 (19), 0.72 (23), 0.73 (18), 0.74 (12). 0.75 (9). 0.76 (2), 0.77 (3). For 100 common pigeons: 0.65 (1), 0.69

(3), 0.70 (4), 0.71 (11), 0.72 (20), 0.73 (13), 0.74 (20), 0.75 (9), 0.76 (5), 0.77 (3), 0.78 (6), 0.79 (3), 0.81 (1), 0.83 (1).

When we examine the quotients obtained from birds whose fast was started in the morning, instead of the evening, a much less uniform picture is presented, particularly among those birds which were measured at 20° C. In these last-named cases, where the rate of combustion (heat production) is shown to have been very high, low quotients were uniformly found from the twelfth to the seventeenth hour. Thereafter, somewhere between the seventeenth and twenty-fourth hours, there is a small but definite rise, which is at once followed by lower quotients. In these cases a maintenance at low temperature, and a starting of the fast at a time of minimum food reserves, combine to modify notably the course of the respiratory quotient between the twelfth and twenty-fourth hours. Where the fast is started in the morning but the combustion is held at a lower plane, by maintenance at a higher temperature (30° C.), the quotients are likely to be high from the tenth to the seventeenth hour, or even later. Here again one finds a slow rate of digestion, and indications of a low reserve or storage of carbohydrate, in these animals.

Four "winter" measurements were made of respiratory quotients 1–7 hours after food. Doves were fasted 24 hours to insure fat quotients and then given 5 grams of food. In one case (run at 20°), a quotient of 0.84 was obtained at 1–4 hours, and 0.88 at 4–7 hours after food; another dove gave a first quotient of 0.84 and a second of 0.81. Two doves (run at 30°) gave quotients of 0.83 and 0.74 at 2–6 hours after food. Thus in one of 4 tests the quotient was unaffected during the first 6 hours that food was present in the crop.

It can be concluded that a 24–hour period without food insures essentially fasting quotients in both doves and pigeons; and that it is not safe to begin metabolism experiments prior to the twenty-fourth hour, if one is to assume a fasting respiratory quotient and measure the carbon dioxide production only, calculating the heat production from the carbon dioxide produced.

Time Relations in The Total Metabolism after Withholding Food

In the series of "summer" tests we also computed the heat production from the known calorific value of carbon dioxide at the determined quotient. It was thus possible to compare the heat production of the same bird at different lengths of time after food. Our study of the values thus obtained, and of the many curves constructed from them, leaves no doubt that usually, though by no means always, the higher quotients

were accompanied by higher rates of heat production. But a really satisfactory method of presenting these results has not been found. We think, however, that this situation is best presented and discussed in connection with the data obtained in the "winter" series of tests.

When one continuously measures the heat-production of a dove or pigeon over any long fraction of a day, a maximum number of difficulties is encountered. In part these difficulties arise from the fact that the birds, common pigeons particularly, are active and restless during daylight hours; in part also from the high probability that the metabolism of these animals is notably reduced during the latter part of the night. It has long been known that pigeons and other birds show a minimum body temperature at about 4:00 A. M. Riddle[4] has shown that both the body temperature and the blood pressure markedly decrease in ring doves between the hours 1:00 A. M. and 5:00 A. M. In a survey of the data of Tables V and VI these sources of confusion should be kept in mind.

The ring doves (Table V), when run in perfect darkness as was done in these cases, are but slightly more active by day than by night. Hence they supply more reliable data on the point now under consideration than do the pigeons. In these tables the heat production is expressed in small calories per 150 grams (doves) or per 300 grams (pigeons) body weight per 4 hours. It will be observed that between the twelfth and twenty-seventh hours these doves show the higher rates of heat production largely coincidentally with higher quotients, particularly in those birds whose period of fast was begun in the evening. These cases show further that between 21 and 27 hours after a fast is thus begun, the heat values obtained can be duplicated in successive measurements. With the common pigeons and scragglies (Table VI), where the difficulties are at a maximum, it is possible to find little relationship between quotient and heat production. The degree of activity which accompanied each measurement is expressed in these tables by roman numerals. Here I indicates minimum and III maximum activity. The more reliable data from the ring doves warrant the conclusion that there is a tendency to an association of high quotients with a high rate of heat production, particularly in the well-fed birds whose period of fasting was begun in the evening.

It is next necessary to compare the total metabolism at about the twelfth hour with that obtaining at 24–30 hours of fasting. Here again we think the data from the ring doves (particularly those whose fasting period

[4] Riddle, O., *Biol. Bull.*, 1908, XIV, 328.

began in the evening) the more reliable. These data show that the metabolism is distinctly lower at the longer interval, but they also demonstrate the futility of making measurements at any time between the twelfth and twenty-first hours after food. This period in these birds presents increases, and irregularities, and high quotients, which are fairly comparable with those that may be encountered in man during the first to the twelfth hours after food. It would seem as necessary to measure the dove's metabolism 24 hours after this type of fasting as to measure that of man 12 hours after food—and for quite similar reasons.

Our several and varied tests indicate that the basal metabolism of the dove and pigeon is essentially that obtaining 24 hours after the last food. This conclusion presupposes other standard conditions stated elsewhere.

The Influence of Prolonged Fasting

In addition to the 24-hour fasting experiments, observations were also made on birds which fasted 48, 72, and in some instances 96 hours. Although comparison of the metabolism of the same bird on different fasting days is difficult, especially if there are pronounced differences in activity,* the results are nevertheless worthy of record. These are accordingly given in Table VII for the common pigeon and in Table VIII for the ring doves. The first metabolism measurements were made 24

TABLE VII

HEAT PRODUCTION PER 300 GRAMS OF BODY WEIGHT PER 4 HOURS DURING PROLONGED FASTING —COMMON PIGEONS

Bird number	Heat production after fasting			
	24 hours (calories)	48 hours (calories)	72 hours (calories)	96 hours (calories)
X233	6895(?)	6118(?)	5324(II)	4332(I)
X250	9630(II)*	7852(II)	6797(II)	———
V762	6594(II)	6405(I)	6359(I)	———
X377	7322(II)	7081(III)	———	———
X17	4864(II)	4747(II)	———	———
Average	7061	6441		

* An egg ovulated into the body cavity at or near this date. This bird never ovulated except into body cavity.

* The birds considered in this section, although measured in the dark, were studied in the daytime. In our more recent work we have found that the ideal time for securing quiet conditions for metabolism measurements is during the night.

hours after the doves and pigeons had been fed 5 and 10 grams of food respectively, by hand. At this time numerous experiments, already described, show that the respiratory quotient approximates that characteristic of fat combustion, and the total heat production is at a level which may be considered the standard metabolism.

The birds were all losing weight during these prolonged fasts, and hence the actual heat production of the bird, considered as an individual, is in practically all cases lower as the fast progresses. But in addition there is a decrease in heat production per unit of body weight. Thus with the common pigeons, the heat production in all instances fell off between the twenty-fourth and the forty-eighth hour of the fast, on the average from 7061 to 6441 calories, or nearly 9 per cent. With pigeons X233 and X250 this decrease was pronounced; with pigeons V762, X377, and X17, the change was slight. Little satisfaction can be obtained by attempting to correct these values for differences in activity, and the decreases noted in the metabolism probably represent real changes. With three of the pigeons (the first three recorded in Table VII) it is possible to compare the metabolism at the twenty-fourth, forty-eighth, and seventy-second hours of fasting. Of these birds the first two show a marked decrease in metabolism at the seventy-second hour, especially X250. With V762, on the other hand, the metabolism 72 hours after food is only 3.5 per cent lower than it was 24 hours after food. With pigeon X233 the fast was continued for 96 hours, when there was a pronounced decrease from 6895 to 4332 calories. It is clear from these comparisons that prolonged fasting results in a distinct lowering in the metabolism of pigeons, an effect in full conformity with that noted with humans during prolonged fasting.

A comparison of the heat production of the different birds is perhaps permissible. Even in this small group of pigeons there is a range in the heat values of from 9630 to 4864 calories in the period 24 hours after food,* and a range of from 7852 to 4747 calories in the period 48 hours after food, the extremes appearing with the same pigeons in both instances. But it should be observed that the careful autopsies performed on these birds soon after the metabolism measurements revealed the quite abnormal condition of bird X250 which gave the extremely high values. An ovum (yolk) had been set free into the body cavity and was being resorbed during these tests. It is thus known that the values obtained here were not representative of the normal life of the bird, though possibly the three fasting values shown here are comparable with each other.

* Some birds, however, were measured at high and others at low temperatures.

A larger number of observations was made with ring doves than with the common pigeons. Since all of the doves were measured both at the twenty-fourth and the forty-eighth hour of fasting, it is justifiable to compare the average metabolism at these two intervals after food (Table VIII). Thus, the average heat production at the twenty-fourth hour is 3934 calories and at the forty-eighth hour only 3343 calories, or 15 per cent lower. In a majority of cases the kymograph records of activity indicated that the dove was quiet, as is common with these small birds

TABLE VIII

HEAT PRODUCTION PER 150 GRAMS OF BODY WEIGHT PER 4 HOURS DURING PROLONGED FASTING —RING DOVES

Bird number	Heat production after fasting			
	24 hours (calories)	48 hours (calories)	72 hours (calories)	96 hours (calories)
Z539	4354(II)	4915(I)	5261(II)	5259(II)
Z57	4544(I)	4380(I)	4387(I)	3576(II
Z612	5831(I)	4288(I)	———	———
Z256, Z608	5375(I)	3757(II)	3207(III)	———
Z592, Z560	4140(II)	3889(I)	3380(II)	———
X125, Z8	3734(II)	3944(II)	3412(I)	———
Z433, Z502	3160(II)	3103(I)	———	———
Z53	3959(II)	2413(I)	———	———
W875	3255(II)	2484(I)	———	———
W866	3983(I)	3642(II)	———	———
W859	3840(I)	2823(I)	———	———
Z707	3386(II)	3149(III)	———	———
Z668	3587(I)	3151(III)	———	———
Z980	3904(II)	3891(I)	———	———
Z238	3936(I)	2799(I)	———	———
T603	2632(II)	3226(II)	———	———
Z47	5318(I)	4216(II)	———	———
Z434	4451(I)	4150(II)	———	———
Z648	4102(II)	2623(I)	———	———
Z861	4209(I)	2282(I)	———	———
Z215	3219(I)	2731(I)	———	———
Z801	2996(I)	2676(I)	———	———
T623	3985(I)	3182(I)	———	———
Z614	2916(II)	3267(I)	———	———
W856	3814(II)	3102(III)	———	———
Z22	3654(I)	2833(I)	———	———
Average	3934	3343		

On the other hand, in a number of instances activity III was noted. Even when activity III prevailed, however, this increased activity was not sufficient to offset the decrease in metabolism caused by the fasting. In four of the experiments pairs of doves were studied together, but in these tests the metabolism has likewise been calculated per 150 grams of body weight. In these four experiments the activity records are somewhat more difficult to interpret; either or both of the doves may have been active. In the more prolonged fasts the comparison can be based only upon the data for each individual dove. The few data, except for the anomalous record of bird Z539, show considerable decrease in the metabolism after the forty-eighth hour; the data obtained at 72 and 96 hours are, however, too scant to deserve comment.

In general, the data make it evident that *prolonged* fasting results in a distinct lowering in the metabolism of both doves and pigeons.

Conclusions Regarding the Use, as Standard Comparable Values, of Metabolism Measurements Made on Pigeons and Doves at The Twenty-fourth Hour of Fasting

We have considered a possible criticism founded on the view that the metabolism of pigeons and doves, measured at the twenty-fourth hour of fasting, is abnormally low for comparison with the metabolism of other large animals or with the metabolism of man measured at the twelfth hour of fasting. The reason for this low metabolism, on this view, is that these small animals have an intense metabolism, hence draw rapidly upon their body stores, and in 24 hours reach a stage of fasting corresponding to a more prolonged fast in man and other animals. Against this argument are the facts that the metabolism of these birds, when quiet, is not intense (as will be brought out later in this report), that doves and pigeons digest and absorb ingested food much more slowly than does man, and that fat quotients are not obtained with these animals until at or near the twenty-fourth hour of fasting.

One may therefore conclude that in the dove and pigeon the metabolism prevailing at twenty-four hours after the ingestion of food has not been profoundly lowered either by undernutrition or by pure fasting. Certainly, any possible change in metabolism induced here by fasting *per se* is not such as to affect seriously the value of the basal metabolism measurements with these birds in comparative physiology; and the error in computing the surface area may far exceed any possible error of comparing the metabolism of pigeons measured twenty-four hours after food

with that of man measured twelve hours after food. We have found definite evidence for utilizing, as the standard condition for basal metabolism measurements with pigeons or doves, a period beginning 24 hours after the last food. This necessarily assumes that the fasting shall begin at the end of the day, immediately following a period of food intake, and that at the hour the fast is begun the bird's crop contains 5 grams (doves) or 10 grams (pigeons) of mixed grain, and a nearly equal amount of water. It presupposes also that this food is not regurgitated, and that during the fast the bird is not extraordinarily active, and is maintained at a temperature of 20°–30° C.

Values Obtained With Different Types of Apparatus

Three types of apparatus have been used in our preliminary studies, and it is possible and necessary to compare the values obtained from these three types of apparatus. As already noted, the multiple (or 10–bird) apparatus is not equipped with means for recording activity, and records of high activity can not be discarded; hence the values obtained with this apparatus, particularly with the more active common pigeons, may be expected to be higher than measurements made with other apparatus. Perhaps a sufficient amount of data is provided in Table IX to permit this comparison. In the 5 upper rows we have tabulated only data for those races which were studied in *all of the three types of apparatus*, in day runs, at 30° C., and after wholly comparable 24-hour preparatory periods. Below, however, are placed data for all races on which more than one test was made with the multiple (10-bird) open-circuit system, together with any other data obtained with either of the other two types of apparatus. We have earlier noted that each of our several races of doves, and each of our several races of common pigeons, must at present be considered apart from other races.

An examination of these data permits the conclusion that, in work with ring doves under the conditions noted, the "10-bird" system and the closed-circuit apparatus give very similar results. Possibly, the 1-bird open-circuit system gives somewhat lower results, due to the elimination of values accompanied by considerable activity. In work with common pigeons, however, it is clear that the multiple apparatus gives results that are much too high. The average and mean values obtained with it were higher in every case than the eight values obtained in work with a 1-bird system. The four groups of measurements, for which the "racial mean" is calculated in this table, show an excess of 26.4 per cent. A large amount of activity that can be neither recorded nor eliminated in work

with this multiple apparatus doubtless provides the explanation of this difference, since these measurements were all made in daytime when activity is highest. It is not surprising that the ring doves fail to show a simi-

TABLE IX

HEAT PRODUCTION AS MEASURED IN DAY-RUNS AT 30°C. BY THREE TYPES OF RESPIRATION APPARATUS

Race	Types of apparatus and values obtained						
	Open-circuit (10-bird)			Open-circuit (1-bird)		Closed-circuit (1-bird)	
	Number of tests	Number of birds	Average calories	Number of tests	Average calories	Number of tests	Average calories
Ring Doves							
63	7	70	3623	8	3448	10	3625
62	2	20	3281	4	3303	5	3532
72	4	40	3530	4	3375	8	3641
N2	5	46	3696	5	3352	4	3769
51	1	10	3437	2	3569	1	3459
Racial mean	(5)		3511		3409	(5)	3605
63 (yg.)	2	20	4209*			4	4114
N2 (yg.)	3	30	3926	1	4246		
51 (yg.)	4	40	3562			4	3751
Common pigeons							
M	5	44	6581*			1	4956
R	2	15	5702	2	5295		
133	3	23	6555			2	4825
R×M	4	40	6840			2	5481
M×299	2	20	6856			1	5958
Racial mean	(4)		6708			(4)	5305

* In these groups not all measurements were made under identical conditions.

Note. Values given are small calories per 4 hours per 150 grams (doves), or per 300 grams (pigeons) of body weight.

lar amount of activity in view of other observations made upon them; they are more quiet under all conditions and in *any* apparatus than are the pigeons. On the other hand, our experience has shown that all these birds are more or less restless in the light, and the 10-bird apparatus provides a more perfect and continuous darkness than does either of the other two types of apparatus.

The Temperature Factor in Metabolism Measurements

It has been demonstrated that lower rates of heat production are obtained from birds measured at 30° than at 20–24° C. Our data concerning two or three aspects of the temperature factor are presented in the accompanying tables and paragraphs.

Measurements and Fasting Periods at 20° and 30° C.

Table X provides a summary of measurements made in day-runs on ring doves at 21-25°, and again at 30° C. Some of the birds measured at 30° had spent the 24-hour preparatory period also at 30° while others had passed this period at ordinary temperatures. Moreover, some of the birds in both temperature groups may have begun the 24-hour fasting period without food in their crops. These variables undoubtedly introduce some irregularities into the results as summarized. In only a few cases was the *same* bird measured at the two contrasted temperatures; age and race differences therefore require that the tabulation be given its present form. The measurements at 22.9° show, in the 12 pure races, an average value 12.2 per cent higher—and a mean value 13.2 per cent higher— than the measurements made at 29.9°. The much smaller numbers of "hybrids" yield a much smaller average difference in the same direction; the mean values obtained from the few hybrids are practically identical. Considering these two groups as wholes it is clear that the factor of age scarcely enters or differs at all. The observed differences may be considered differences resulting from the temperature difference. This is, of course, subject to the circumstance that not the same individuals, but individuals of the same race, are here compared; further, the values measured are from day-runs, which give values somewhat higher than those of night-runs. Nevertheless, these data strongly suggest that lower values are obtained at 30° than at temperatures only a few degrees lower.

In determinations made at night, after standard preparatory periods and using the same bird in measurements at the lower and higher temperatures, we have obtained our most reliable data on the influence of the temperature factor. Table XI records the results of these double measurements of 23 birds (for natural groups, or classes). Values obtained at 20–22° average 17.9, 17.3, 17.4 and 15.1 per cent higher than at 30°, a decrease of about 2 per cent in the metabolism per degree C. In three individual cases the kymograph records of the two determinations on the same bird were not equivalent, and these cases are noted on the table. These data indicate that when the metabolism of either doves or pigeons

TABLE X

METABOLISM AT OR NEAR 23°C. AND AT 30°C. MEASURED (DAY-RUNS) ON THE SAME RING—DOVE RACE, OR ON HYBRIDS OF THE SAME CROSS

Race	Exact Temp. of Exp.	Number birds used	Average Age	Average Calories*	Cross of races	Exact Temp. of Exp.	Number birds used	Average Age	Average Calories*
			months					months	
	Pure races				Hybrids				
63	29.6	23	16.6	3468	1×51	29.9	7	15.7	3570
	23.5	7	13.1	3437		23.8	6	19.1	3914
N2	29.8	9	19.6	3537	72×63	30.0	2	10.1	3456
	22.4	3	17.5	4895		22.5	1	8.9	3383
72	29.9	13	20.8	3545	61×117	30.2	2	15.7	3406
	23.3	8	17.0	4065		23.7	1	18.9	3685
62	29.5	10	21.9	3361	11×62	29.7	2	17.3	3547
	22.9	5	21.4	4054		22.3	1	18.6	3778
51	29.2	3	17.8	3533	50×63	30.3	4	16.3	3582
	24.3	2	25.6	3537		23.4	2	17.7	3188
NE	30.8	4	21.3	3697	72×44	28.2	1	13.0	3517
	21.8	2	12.2	4457		24.2	2	14.3	3708
44	30.1	1	17.6	3643	44×N2	29.5	3	17.0	3622
	21.0	1	12.4	4344		25.1	2	19.2	2964
50	29.8	6	18.2	3203					
	21.3	2	22.7	4252					
NA	30.0	5	18.9	3792					
	22.7	2	14.4	4084					
11	29.6	4	18.1	3324					
	23.4	1	31.6	3272					
67	30.0	6	22.9	3896					
	22.3	2	12.7	3971					
OT	30.2	1	12.0	3008					
	23.1	2	19.7	4044					
Racial means	29.9	12	18.8	3501		29.7	7	15.0	3529
	22.7	12	18.4	4034		23.6	7	16.7	3517
Average	29.8	85	19.1	3508		29.9	21	15.5	3549
	22.9	37	17.4	3996		23.7	15	17.6	3603

* Small calories per 4 hours, per 150 grams body weight.

TABLE XI

METABOLISM AT 22° AND AT 30° MEASURED (NIGHT-RUNS) ON THE SAME INDIVIDUALS.
Birds kept during 24-hour fasting period at 22° or at 30° and measured at same temperature.

Bird number	Exact Temp. of Exp.	Calories*	Bird number	Exact Temp. of Exp.	Calories*
Ring dove races			Ring hybrids		
D856	29.8	3444 (I)	H176	30.1	2548
	20.1	3994 (I+)		22.2	3132
Z610	29.6	2874	D855	30.1	2800
	21.7	3181		22.2	4006
Z839	29.8	2718	D712	29.7	2865
	21.1	3755		22.0	3864
T680	30.1	2763	H174	30.1	3456
	22.2	3477		21.1	3523
H41	30.0	3367	Z596	29.6	2418
	21.8	4137		20.1	3929
D898	29.9	3168	H418	29.9	3775 (I+)
	21.3	4093		20.1	3380 (I)
D978	30.0	2884	D437	30.2	3372
	22.2	3256		22.0	3322
D909	30.0	3201 (I)	D872	30.2	3293
	22.2	3462 (I+)		21.9	4506
Z802	30.0	2618			
	21.8	3577			
Average	29.9	3004	Average	29.9	3066
	21.6	3659		21.5	3708
Common pigeon races			Common pigeon hybrids		
X703	30.1	4956	X779	30.0	5210
	21.0	7722		21.9	5732
X503	29.9	4114	X999	29.7	5464
	22.1	4075		22.1	6567
X945	29.9	5248	J17	29.9	6965
	21.9	5539		21.9	8503
Average	29.9	4773	Average	29.9	5880
	21.7	5779		21.9	6934

* Small calories per 4 hours per 150 grams (dove) or 300 grams (pigeon) body weight.

is measured at 30° the values obtained are 15 to 18 per cent lower than those obtained at 22°.

The possible influence of the external temperature at which the bird is living prior to measurement, particularly during the immediately-preceding 24-hour fasting period, presents another aspect of the problem of temperature. The data shown in Table XII were obtained at all seasons of the year; among these birds therefore some of the 24-hour preliminary

TABLE XII

EFFECTS OF LOW TEMPERATURE, DURING THE 24-HOUR PERIOD PRECEDING MEASUREMENT. Heat production in ring-doves which are not hand-fed at the beginning of the fasting period (day-runs, 30°).

Race	Fasting pen not heated; birds not hand-fed at beginning fasting period		Pen heated (30°); birds not hand-fed	
	Number birds tested	Average calories*	Number birds tested	Average calories
63	5	3184	9	3349
62	9	3212	15	3505
N2				

* Small calories per 4 hours per 150 grams body weight.

periods were passed in winter temperatures as low as 12°, others in summer temperatures reaching nearly 30° C. The results indicate that some ring doves, if not hand-fed at the beginning of the 24-hour fasting period, show a lower metabolism when the 24-hour period is spent in low temperatures than when spent at 30°. In one of the two tabulated groups (Race 63) the indicated reduction is 5 per cent; in the other, this is 8 per cent. Birds with little or no food in the crop at the beginning of the 24-hour fast thus appear to use their food stores more rapidly at low temperatures, and to have reached a more advanced stage of actual fasting when measured 24 hours later.

At a restricted period of the year (May to July) a further study was made to learn whether similar effects of the low temperature during the fasting period are observable in birds which were hand-fed at the beginning of the period. The results are shown in Table XIII. Seven ring doves and 8 common pigeons were twice measured, once after a 24-hour preliminary period at 30°, and again after this period at a temperature nearer 20°. The results of tests made under these conditions, and at this season (when the ordinary external temperatures often or ordinarily lie between 20° and 30° C.), do not show any consistent reduction, perhaps no reduction at all, as a result of exposure to a temperature of 20-23° during 24 hours

TABLE XIII

COMPARISON OF HEAT PRODUCTION MEASURED IN NIGHT-RUNS.

Birds kept during the 24-hour preliminary period at 20° or at 30°C. Small calories per 4 hours per 150 grams (dove) or 300 grams (pigeon) body weight.

	Bird	Temperature (C.)		Calories (and activity)
		During 24-hour fasting period	During measurement	
Ring-doves	H217	30°	30°	3209 (I, I)
		19	30	3244 (I, I+)
	H220	30	30	3212 (I, I+)
		19	30	3000 (I+, I+)
	D789	30	30	2736 (I)
		19	30	2635 (I)
	D724	30	30	3286 (I+, I+)
		19	30	3180 (I, I+)
	H696	30	30	3363 (I+)
		20	30	3349 (I)
	H699	30	30	3356 (I, I+)
		20	30	3259 (I, I)
	H686	30	30	3082 (I, I+)
		20	30	3064 (I, I)
Common pigeons	J138	30	30	6586 (I+)
		23	30	6982 (I+)
	X345	30	30	5847 (I+, I+)
		23	30	4213 (I, I+)
	X715*	30	30	7179 (I, I+)
		23	30	8086 (I+, I+)
	J42*	30	30	6478 (I+)
		23	30	6769 (I+)
	J206	30	30	4972 (I, I+)
		22	30	6548 (I+, I+)
	X560	30	30	3899 (II−)
		22	30	4181 (II)
	X632	30	30	4865 (I+)
		22	30	4997 (II)
	X578	30	30	4492 (I)
		22	30	4649 (II+)

* "Scraggly" pigeon.

preceding the measurement. The average value for the doves is 2.3 per cent less, and that of the pigeons 4.5 per cent greater, (also definitely greater activity) after a fasting period spent at the lower temperature. In earlier paragraphs of this section it has been shown, however, that birds have a higher rate of heat production at 22° than at 30° and are thus using their food reserves at a higher rate. If, therefore, it is desired to measure the metabolism of all birds at all seasons on a comparable part of the fasting level it is necessary not only to start all birds with equivalent food but to conserve equally this food by providing a uniform temperature at which it is to be burned.

From the data presented here we conclude that temperature is an important factor in determining the rate of heat production in doves and pigeons. When the measurements are made at temperatures ranging between 20° and 30° C each degree modifies the metabolic rate by approximately 2 per cent. If measurements made at various seasons are to be made at a comparable part of the fasting level, it is necessary to keep all birds at a uniform temperature during the 24-hour fasting period.

Day and Night Measurements

Measurements Made in Darkened Chamber during Day and Night

Our kymograph records clearly show that both ring doves and common pigeons are quieter during tests made at night even though the daytime measurements are made in a darkened chamber. A considerable fraction of the total number of tests made during daylight hours has had to be entirely discarded because of activity. The amount of loss from this source alone is sufficient to make it inadvisable to attempt to measure the metabolism of these animals during the daytime. But even after excluding these cases of marked activity our data show that notably lower values are obtained at night. The preliminary treatment, including the fasting period, was the same for the two groups.

Table XIV adequately presents the uniformity and extent of the differences still present after eliminating all records showing much activity. In 11 ring dove races, or types of hybrids, the daytime tests show a mean increase of 18.8 per cent over those made at night. Four such groups of common pigeons, measured in the 10-bird apparatus, show a similar difference of 16.6 per cent. Eight groups of common pigeons (in 1-bird apparatus) show a mean difference of only 7.9 per cent; but the racial means in this case are unduly influenced by the very meager and wholly excep-

TABLE XIV

COMPARISON OF HEAT PRODUCTION OBTAINED IN DAY AND NIGHT MEASUREMENTS. Usually different individuals, but of the same race, were used in the two tests.

Race	Night		Day	
	Number of tests	Calories	Number of tests	Calories
Ring doves				
63	2	3207	23	3468
N2	2	3092	9	3537
62	1	2744	9	3430
62*	1	3799	4	4129
29	1	3332	1	4053
51	2	2910	3	3533
OT	2	2654	1	3008
11×62	2	2464	8	3549
N2×63	2	3120	5	3732
44×N2	2	3216	3	3622
36×62	2	2974	1	3775
Racial mean	(11)	3047	(11)	3621
Common Pigeons				
R	6	4835	2	5395
133	7	4805	1	4784
294	1	5012	2	6008
294*	2	4742	2	7237
267	2	5167	1	4456
299*	1	7080	1	4864
Scraggly	2	7254	2	8144
PT×272	3	6833	3	8431
Racial mean	(8)	5716	(8)	6165
In 10-bird apparatus				
133 (yg).	1	5841	3	7360
R×M	2	5453	4	6840
M×299	2	6438	2	6856
M×299 (yg).	1	6731	1	7479
Racial mean	(4)	6116	(4)	7134

* Tests made (both columns) at 22–25°C.; all other tests at 30°C.

Note: Values are small calories per 4 hours per 150 grams body weight (dove) or 300 grams body weight (pigeon).

tional data for race 299. Only 2 of a total of 38 measurements were made on this race, and if these are omitted from the calculation the racial mean for the remaining 7 races indicates that the day-runs are 15.0 per

cent above the night-run values. It is evident that measurements made during daytime are approximately 15 per cent higher than those obtained at night. This consideration makes it clear that present and future work on doves and pigeons should all be done at night.

Light and Darkness in Relation to Activity and Heat Production

It has been found that both ring doves and common pigeons show less activity when kept in complete darkness during measurement than when kept in the light. Since the amount of activity when measured in the light is sufficient totally to invalidate a very high percentage of the values obtained, it is evident that all measurements should be made in darkened chambers.

This point is established for ring doves, measured in day-runs, by the data of Table XV. Eight doves were each measured during three successive periods; that is, without at all touching or disturbing the birds during the three periods. During two of these periods the birds were left exposed to the light, while during the other period they were kept in complete darkness. It will be seen that each of the eight "dark" periods was accompanied by a lower grade of activity than that of its two associated "light" periods. Corresponding to this difference in activity the heat production during the periods measured in the dark was, in almost every case, very much less than in the associated periods which were measured with the birds exposed to the light.

All of these results, as well as additional experience, emphasize the necessity of making metabolism measurements of doves and pigeons in complete darkness. At the Nutrition Laboratory there has been constructed a modification of the closed-circuit apparatus for a single bird which is adapted to the simultaneous and separate measurement of four rats or birds and also permits a complete control of light. This apparatus was recently exhibited at the Carnegie Institution of Washington, and is now in use at Yale University and in our own studies.

Other and different tests and observations strongly suggest that, apart from recordable activity, there is a specific effect of light which increases the metabolic rate in these animals. The question is being further studied and the results will be more fully reported at a later time.

EFFECT OF REPEATING MEASUREMENTS AT SHORT INTERVALS

In actual practise it is sometimes convenient, even with a large colony of birds at hand, to repeat measurements of the rate of heat production after the lapse of only a few days. In the earlier stages of this study

TABLE XV

THE EFFECTS ON ACTIVITY AND HEAT PRODUCTION IN RING DOVES OF MEASUREMENTS (DAY-RUNS) MADE WITH BIRDS EXPOSED TO LIGHT

Bird and date	Periods measured	Calories*	Activity	Light or dark	Temperature
Z646	1	4841	II	Light	29.1
I/5	2	3643	I	Dark	28.8
	3	4686	II	Light	28.8
Z507	1	5476	III	Light	30.0
I/5	2	3667	I+	Dark	30.7
	3	5536	III	Light	30.6
D169	1	5130	II	Light	30.1
I/6	2	4942	I	Dark	30.1
	3	5212	III	Light	29.9
T619	1	4622	I+	Light	30.0
I/6	2	3666	I	Dark	30.0
	3	4396	III	Light	30.1
T731	1	3993	II	Light	30.1
I/7	2	4332	I+	"	29.8
	3	3493	I	Dark	29.7
Z610	1	4189	III	Light	30.2
I/7	2	4292	III	"	30.2
	3	3390	II+	Dark	30.5
Z856	1	4541	I+	Light	30.0
I/7	2	4796	II	"	29.9
	3	4614	I	Dark	30.0
D31	1	4044	III	Light	30.0
I/7	2	4245	I+	"	30.1
	3	3168	I	Dark	30.1

* Small calories per 4 hours per 150 grams body weight.

this was sometimes done, but an inspection of the results led to the complete abandonment of this procedure. An interval of at least 21 days now separates any two measurements that we make on any one bird. Our evidence that a period longer than 7 days should elapse between such measurements has been fully tabulated, but it seems possible to present it by a short description.

Ten ring doves were measured (under comparable conditions and in the same degree of repose) a second or third time, in day-runs, at intervals

varying from 3 to 11 days. Seven of the 10 final measurements gave lower values than the corresponding initial measurement. The average decrease in the ten pairs of measurements was 9.7 per cent. A decrease of 9.4 per cent was obtained from four tests with two common pigeons. These results were later essentially confirmed by measurements made at night and at intervals of 7 days. Here a slightly lower average value was obtained from 4 ring doves (0.4 per cent), and from 4 such tests with common pigeons (5.5 per cent). We conclude that in some birds, though not in all, the rate of heat production is somewhat decreased in measurements repeated after an interval of 7 days or less.

A consideration of the explanation of this decreased heat production (in those cases where it occurs) would include reference to the fact that most of the doves and pigeons used by us have only a small reserve of visible fat, that our studies on the respiratory quotient suggest a small glycogen reserve, and that the incidental handling and gentling of some birds in a first measurement possibly results in less activity in an early-repeated test. Whatever the explanation, our results indicate that measurements should not be soon repeated on the same dove or pigeon.

Effects of Previous Inactivity or Close Confinement

The birds used in this study are, of course, confined in cages, and it is necessary to enquire whether their basal metabolism is affected by this restriction on the conditions which would seem to be normal to bird-life. This question can be resolved by a study of common pigeons of our vicinity which live in the open, without restraint; and by placing our own doves and pigeons into much smaller cages than those they normally occupy. In another section it has been noted that all of our birds are given a rather large amount of flying space until sexually mature; thereafter, nearly all of them are placed in cages of smaller size ($0.6 \times 1.5 \times 2.2$ meters; or nearly 2 cubic meters) which nevertheless permit rather free movement and short flights. Digressing for a moment, we note that most of the quite young birds measured by us were living in larger cages than were the mature birds (with which they are compared) and it is not yet certain that the so-called "effects of age," which are discussed in the next section, are due exclusively to age; the larger flying space afforded the young birds may be responsible for at least a part of the higher values obtained for them.

We have not yet had the opportunity to measure the metabolism of pigeons which fly at large, without any restraint of caging or captivity. A test of the opposite kind, however, has been made. In this test pairs of

birds were kept for a minimum of four weeks in very small cages (1 cubic foot, or 28 liters). Their basal metabolism was then obtained. They were then transferred to the cages to which all our birds are accustomed (about 75 times larger than the small test cages). After a minimum of another five weeks their metabolism was again measured. Altogether, 38 acceptable measurements were made on 7 males and 8 females under these two very different degrees of restraint. The results of this test, made in 1927, are given in Table XVI.

TABLE XVI

EFFECT OF CONFINEMENT ON HEAT PRODUCTION.

The rate of heat production in birds after four to five weeks of inactivity, or very close confinement, compared with that obtained after living for a similar term in much larger cages.

Sex	Number of tests	Calories per 4 hours (per 150-gram bird) in:	
		Small cages (1 cubic foot)	Larger cages (75 cubic feet)
♂	2	2861	2974
♂	4	3132	3131
♂	2	3300	3639
♂	2	2844	2936
♂	4	3005	4339
♂	2	3868	4404
♂	3	2855	3268
♀	3	3240	3235
♀	2	3086	3284
♀	2	3420	3533
♀	2	3038	3513
♀	2	3103	3384
♀	3	3043	3876
♀	2	3845	4055
♀	3	3279	3215
Average		3195	3586

The data of the table show that the 28–35 days of close confinement resulted in lowering the average basal metabolism of 15 birds by 10.9 per cent. The birds were slightly older when tested in the larger cages. All measurements were made between April 28 and July 12. We think the differences found are attributable to no other factor than that of inactivity or close confinement. This result is similar to that which Lusk[5] has reported for a dog. It was doubtless also to be expected in the pigeon since Riddle and Honeywell[6] had shown that three to five weeks

[5] Lusk, G., *Jour. Biol. Chem.*, 1915, xx, 565.

[6] Riddle, O. and H. E. Honeywell., *Amer. Jour. Physiol.*, 1924, LXVII, 333.

of close confinement reduces the normal amount of sugar in the blood of pigeons by nearly 30 per cent.

It is of some interest to note the further special result that the males seem to have been more affected by the close confinement than were the females. If we consider the metabolism in larger cages as the normal, this normal was reduced in the males by 14.9 per cent, while the females lost only 7.3 per cent. Whether the sexes do in fact respond to a different extent to this factor is a question which is being tested further. Meanwhile it must be concluded that doves confined in very small cages, for a period certainly as short as four weeks, may not be expected to have a normal basal metabolism; their normal "basal" has been diminished.

The Age Factor

The great majority of birds used in this study were adults, aged 10–25 months; but in any extensive study the measurement of birds of varying age is unavoidable. It is therefore necessary to know whether, and to what extent, the results are affected by the age factor. For a survey of this point it was decided to make measurements of a considerable number of immature birds (usually 3–6 months old) and to compare the values obtained from them with values from the birds of more advanced age. By adult birds we mean birds that are sexually mature, and have remained for at least three weeks in breeding pens of standard size (nearly 2 cubic meters). The immature birds used in our measurements all had attained practically their adult size (which occurs at or before 3.0 months), they were not sexually mature (this certain only for females), and they were still living in cages considerably larger than the standard breeding pens.

Table XVII records the comparisons possible from all measurements made in day-runs at 30°C. on birds fasted 24 hours. Otherwise, the standard preparatory period was not maintained on some of these birds. In 12 of the 14 races, or groups, the higher rate of heat production is found in the immature birds. In race 62 exactly the same value (3520 calories) is found in the young as in a single measurement of adults. In one other race (72) the young show a smaller value than was obtained in a single measurement of adults. On the average the young show a metabolic rate 9 per cent higher than that found in the adults. Ring doves and common pigeons show a quite similar relation of the age factor to heat production.

We do not assume that this comparison of immature with mature doves and pigeons constitutes an adequate study of the age factor. However, our continued investigations, without special effort, will later

TABLE XVII

THE EFFECT OF AGE ON THE RATE OF HEAT PRODUCTION (DAY-RUNS AT 30°C.)

Race	Adult			Young			Per cent excess in young
	Number of tests	Age	Calories*	Number of tests	Age	Calories*	
		months			months		
Ring doves							
63	23	16.6	3468	4	4.8	4114	
51	3	17.8	3533	4	4.3	3751	
Racial mean	2	17.2	3501		4.6	3933	+11.0
Common pigeons							
299	2	21.2	5728	2	9.5	6776	
Scraggly	2	15.9	8144	1	5.5	9179	
Racial mean	2	18.6	6936		7.5	7978	+13.1
With 10-bird apparatus							
Ring doves							
63	6	16.1	3626	2	5.6	4209	
51	1	23.2	3437	4	3.6	3562	
N2	5	20.5	3696	3	4.6	3926	
62	1	18.2	3520	5	4.4	3520	
72	1	23.9	3533	2	4.0	3342	
Racial mean	5	20.4	3562		4.4	3712	+4.0
Common pigeons							
M	5	18.0	6581	4	5.3	7250	
R	2	16.6	5702	4	4.3	6354	
133	3	17.4	6555	3	5.9	7360	
R×M	4	12.8	6840	5	4.0	7253	
M×299	2	12.5	6856	1	3.2	7479	
Racial mean	5	15.5	6507		4.5	7139	+8.9

* Small calories per 4 hours per 150 grams (doves) or 300 grams (pigeons) body weight.

provide data sufficient to permit further and conclusive comparisons between mature birds aged 10 months with others aged 20 or 30 months. In that study all birds will have been taken for measurements from cages of identical size. Our kymograph records do not indicate that the young birds are more inclined than older ones to activity during measurement.

We conclude that immature but full-grown doves and pigeons have higher rates of heat production than they have at older ages, and that this is probably a true age effect. Age records are therefore significant and necessary to the use and interpretation of measures of the basal metabolism of these animals.

Summary and Conclusions

Several physiological factors or conditions which were conceivably capable of modifying the basal heat production of doves and pigeons have been studied and are found to influence the values obtained from these animals. The elimination or the control of these factors is essential to success in measuring such differences as may exist in the basal metabolism of the sexes, of different races, and of different hybrids, among these birds.

A statement is made concerning the special nature and history of the wide variety of races (24 of doves; 13 of common pigeons) and hybrids upon which these and current studies are based. The dove and the pigeon, by their accepted classification, differ more than the rat and the mouse; and the distinctness of at least some of the many races of each of these species makes it necesssary to treat and tabulate data with full recognition that they are different races or strains. More than a thousand birds of known pedigree, maintained under excellent conditions, have been continuously available for this study. Autopsies made usually soon after our metabolism measurements enable us to eliminate from consideration all values obtained on diseased birds.

Our study of the time relations of the respiratory quotient shows that a 24-hour period following the taking of standard amounts of food insures essentially fasting quotients (about 0.72). It is not safe to begin metabolism experiments prior to the twenty-fourth hour, if one is to assume a fasting respiratory quotient and calculate the heat production from the carbon dioxide production only.

The fasting period should begin near the end of the daylight hours—immediately following the hours of normal feeding. At this time the crops of ring doves should be provided (hand-fed, when necessary) with 5 grams of grain; those of common pigeons with 10 grams, to which water

is added. In this way a favorable stage of fasting will be obtained 24 hours later, and the end of the fast then coincides with the most favorable time (night) for making metabolism measurements.

Some birds not hand-fed at the supposed beginning of a fasting period will be measured in a fast actually much longer than 24 hours and will yield values lower than normal 24-hour values. Unless precautions are taken some birds will regurgitate food which will again be eaten at a time nearer the period of measurement; this will result in an increse in both the respiratory quotient and the metabolism.

The effect of the fasting is increased if the birds are held at low temperatures, or if fighting or other marked activity is permitted, during the 24-hour fasting period. The fasting period must therefore be made a general preparatory period in which food, temperature and activity are fully equalized and controlled in a specially constructed cage.

It is found that prolonged fasting results in a distinct decrease of the metabolism in both the pigeon and the dove.

Measurements not made with the bird in approximate repose should, of course, be rejected. In addition, it is found that to avoid any "after effect" of activity or excitement of the bird during its transfer to the respiratory chamber the measurement should begin only one hour after the bird is placed in the chamber.

Temperature is an important factor in determining the rate of heat production in doves and pigeons. Between 20° and 30° C. each degree modifies this rate approximately 2 per cent.

Light must be excluded from the bird while in the respiratory chamber. These birds show a greater tendency to movement in the light than in the dark, during both day and night. In addition, it is quite possible that light here has a specific effect in increasing the metabolic rate.

Measurements should be made exclusively at night, the normal period of repose for these animals. Tests made at 30° C. during the daytime, in darkened chambers into which light is passed only at intervals for temperature readings, yield values approximately 15 per cent higher than those obtained at night in continuously darkened chambers. Truly "basal" values from doves and pigeons are to be sought among measurements made at night in completely darkened chambers. The hours from 1:00–5:00 A.M. should probably be avoided, since especially low body temperatures and lowest blood pressures have been found to occur in these animals at this time.

Our tests have indicated that measurements necessarily accompanied by fasting periods should not be soon repeated on the same bird. Most birds, though not all, will show a lower heat production in a second measurement which is made within 7 days of an earlier test.

Prolonged inactivity and close confinement prior to measurement markedly decrease the metabolism. Doves confined in very small cages, for a period certainly as short as four weeks, do not then have a normal basal metabolism; their normal "basal" has been diminished.

Immature but full-grown doves and pigeons have somewhat higher rates of heat production than birds at older ages; this is probably a true age effect.

A study of the factors which modify the rate of heat production in doves and pigeons has demonstrated that such factors exist in very considerable number and complexity. It is thought that this investigation of these factors, together with the development of the apparatus and technique described in the preceding paper, now makes it possible to obtain reliable measures of the basal metabolism of these animals.

VITAMIN REQUIREMENTS OF NURSING YOUNG

VII. THE PRODUCTION OF UNCOMPLICATED VITAMIN-B DEFICIENCY IN NURSING YOUNG OF THE ALBINO RAT*

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OUR recent investigations in connection with vitamin B deficiency of nursing rats concerned themselves with complicated avitaminosis, that is, the diets employed were deficient not only in vitamin B but also in vitamin G. In this communication we are submitting the biological technique of producing uncomplicated vitamin B deficiency of nursing young of the albino rat, which is as follows:

Lactating mothers are transferred on the date of the birth of their litters, reduced to 6 in number, from stock diet 1 (1) to ration 1009,(2) deficient in vitamins B and G,[1] and the mothers are given a supplementary daily adminstration of 500 mg. of dehydrated Northwestern yeast separately from the ration. Mothers and young are weighed daily, and daily records are kept of food consumption. The first objective is to prevent appreciable losses in the weight of mothers, which is accomplished by the supplementary yeast allowances. The next step is to induce sufficient growth in the nursing young, so that a litter of 6 will attain a collective weight of 120 to 130 grams. In order to do this, it is frequently necessary to increase the daily portion of dehydrated yeast to 750 mg. The increase is made when the litter shows slow gain in weight 14 to 16 days after birth. We then encounter either a plateau curve in the nurslings or slow gain, and at this time ration 1009 is changed to ration 1438, which is abundant in vitamin G, but deficient in vitamin B. This change is made by replacing 10 per cent of the dextrin in ration 1009 by an equivalent amount of dehydrated yeast, autoclaved for 6 hours at 15 to 18 pounds pressure. The supplementary daily allowances of dehydrated yeast to the lactating mothers are now discontinued. Such a procedure insures the secretion of an abundance of the stable factor in the milk, and the

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[1] In accordance with the recommendation of the committee on Vitamin B terminology appointed by the American Society of Biological Chemists. (See *Science*, 1929, LXIX, 276).

avitaminosis subsequently developed in the nursing young is uncomplicated vitamin B deficiency. After this change in the maternal diet is made the young first respond to vitamin G, but finally reach a state of prolonged maintenance. During the later period of vitamin B depletion the mothers very frequently lose from 20 to 25 per cent of their body weight, and at a critical point further loss of weight in the mothers is prevented by the administration of daily small dosages (20 to30 mg.) of concentrated extracts of vitimin B.[2] The young eventually develop posterior paralysis, labored respiration, cyanosis, and finally death occurs, unless vitamin B therapy is instituted. Table I offers a typical illustration. It will be noted that on November 1st, a day after the three remaining young began to receive vitamin therapy although the nurslings responded in growth, the mother was in an advanced state of polyneuritis. On that day she showed not only paralysis, but also chills, and cyanosis. The fact, however, that she was not obliged from now on to furnish vitamin B to her litter, enabled her with the 20 mg. daily dosage of yeast concentrate No. 26 to regain her well-being in as short a period as 48 hours.

Recently we have found that, if ration 1438 is further modified, so as to contain 5 instead of 10 per cent autoclaved dehydrated yeast, and the mother is allowed a supplementary daily portion of 750 mg. of the same yeast, so as to insure the secretion of an abundance of the stable factor in the milk, the accentuated condition of polyneuritis is expedited, and we then encounter a collapse 7 to 10 days earlier. From such results we conclude that a ration furnishing as much as 10 per cent autoclaved yeast must contain sufficient undestroyed vitamin B to keep the nurslings that partake of the maternal diet at maintenance for a considerable length of time. We, therefore, vary our technique to produce the various desired phases of the avitaminosis in the nursing young, in order to study the biochemical changes in the blood and tissues.

To date we have examined a total of 80 nursing young suffering from uncomplicated vitamin B deficiency and on post-mortem examinations such nurslings revealed the same character of hemorrhages as found previously in baby rats suffering from a deficiency of the vitamin B complex (3). In addition, we observed severe hemorrhages in the vertebral column. We also detected in the majority of the pathological young, hypertrophy of the heart, particularly the right auricle, which is in harmony with the recent findings of McCarrison in avian beriberi (4). The pathological

[2] In this study we used yeast concentrate No. 26, prepared by Mr. E. H. Stuart, chemist of the Eli Lilly Research Laboratories, by fractional precipitation with alcohol.

TABLE I.

THE PRODUCTION OF UNCOMPLICATED VITAMIN B DEFICIENCY IN NURSING YOUNG OF ALBINO RAT

Lactation Record of Female 5428* (Second litter) Transferred from Stock Diet 1 on Date of Birth of Litter to Ration 1009, Deficient in both Vitamins B and G.

Date (1928)	Female 5428	Litter	Food consumed during previous 24 hours	Date (1928)	Female 5428	Litter	Food consumed during previous 24 hours	Remarks
Sept.	Gm.	Gm.	Gm.	Oct.	Gm.	Gm.	Gm.	
14	226			10	149	139	18	* Female 5428 had a
15	178	(1) 28		11	148	147	21	litter of 8 young and
16	181	32	10	12	148	147	21	was allowed to rear 6.
17	180	35	13	13	145	147	16	
18	176	40	13	14	142	150	20	
19	175	44	14	15	138	151	15	
20	169	49	13	16	130	149	20	
21	167	55	14	17	140	150	20	
22	163	60	16	18	138	153	16	
23	166	64	16	19	138	151	18	
24	167	73	19	20	136	152	16	
25	166	78	18	21	133	153	16	
26	167	87	23	22	134	153	18	
27	168	93	21	23	136	155	17	
28	169	100	23	24	133	155	17	
29	165	107	21	25	132	155	14	
30	165	(2) 110	24	26	128	152♯	16	
Oct.				27	126	151	14	
1	168	121	25	28	125	151	15	— Young eating.
2	164	123	21	29	126	(4) 147	15	
3	162	126	20	30	130	137♯♯	18	♯ Beginning to drag
4	162	(3) 127	17	31	138	82 (3)§	20	hind legs
5	159	131	19	Nov.				♯♯ Marked posterior
6	155	135	16	1	138§§	92	23	paralysis
7	155	137—	18	2	138†	101	29	
8	153	135	14	3	148‡	111	28	
9	149	134	15	4	146	115	22	
				5	153	120**	22	** Young weaned.

(1) Lactating female received a daily supplementary administration of 500 mg. of dehydrated yeast (Northwestern).

(2) Supplementary daily administration of dehydrated yeast increased to 750 mg.

(3) Ration changed to No. 1438, deficient in vitamin B, but abundant in vitamin G. Supplementary daily allowance of yeast to mother discontinued.

(4) Mother given a supplementary daily administration of 20 mg. of yeast concentrate No. 2.

§ Number in parenthesis following weight records of nurslings indicate how many young were left on that day. The remaining 3 young showed advancing symptoms of polyneuritis on Oct. 31 and were given vitamin B therapy; 60 mg. of a rice polish extract was given daily to each young.

§§ Mother in a very poor condition; posterior paralysis; muscle chills, and cyanosis.

† Animal's condition seems improved.

‡ Animal much improved; no signs of polyneuritis.

changes in the liver are characterized by capillary congestion, hemorrhage and atrophy (5). Out of 44 animals investigated 17 showed a progressive form of hypoglycemia, and 27 an accentuated hypoglycemia in the premortal state. Anhydremia, associated with disturbance in hematopoietic function has also been found in such polyneuritic nursing young (6). Such baby rats also showed a marked reduction in the glycogen content of the liver (7). The constipated condition we found in the majority of the polyneuritic baby rats is in agreement with similar findings in infantile beriberi recently reported by Hoobler (8).

The pathological nursing young responded readily to vitamin B therapy. This was accomplished by daily administrations of concentrated extracts from rice polishings. As little as 12.5 mg. daily of our extract of rice polishings was found sufficient for continuous growth of nursing young during lactation. In attempting, however, to save nurslings in advanced states of polyneuritis we were obliged frequently to increase the daily dosage to 60 mg. Recently we have succeeded in saving young in the most accentuated form of the avitaminosis when the individuals were in marked opisthotonos,and gasping for breath. Vitamin B therapy produces increase of growth, rapid increase in concentration of blood sugar and a regeneration of blood (6).

Summary

Biological technique is described for the production of uncomplicated vitamin B deficiency in nursing young of the albino rat, and a summary of pathological symptoms in baby rats in such avitaminosis is presented.

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Editorial Review

WHAT PLACE HAVE ALUMINUM, COPPER, MANGANESE AND ZINC IN NORMAL NUTRITION?

IT HAS for a long time been recognized that more than 99.9 per cent of the total weight of the body is composed of an association in different forms of a dozen chemical elements: carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, chlorine, potassium, sodium, calcium, magnesium and iron. It has also been appreciated for a considerable period that traces of other elements, such as aluminum, copper, manganese and zinc, regularly occur, and investigations of the past few years have strongly suggested that the presence of any element should not be regarded as fortuitous without proof, even though its functions have not yet been discovered. Experiences with the physiologically active products of the glands of internal secretion and with the vitamins have impressed anew the importance of what Mendel (1923) has styled the "little things" in nutrition. While it may be that some of the mineral elements discovered in animal tissues are present merely because they have been taken in with vegetable food, and their biological significance is for the plant rather than the animal, nevertheless it behooves us to inquire into their possible rôle as catalyzers of metabolism in the animal body.

It is not the purpose of this paper to treat exhaustively the researches relating to such mineral elements as they cover too wide a field for a single review. Elements which have been found in living plants and animals in addition to those generally accepted as essential to animal life are aluminum, arsenic, boron, bromine, cobalt copper, fluorine, iodine, lithium, manganese, nickel, silicon, strontium, titanium, vanadium, and zinc. Even traces of gold have been recently reported by Berg (1928) in various tissues of plants and animals, including whole grains, fruits and nuts, human blood, ox liver and brain. Boron has been found essential to the growth of the tomato plant by Johnston and Dore (1928) and to that of the broad bean, *Vicia Faba* by Warrington (1926). The Boyce Thompson Institute has reported great improvement in the fertility of the soil in Florida upon the addition of small amounts of copper. Howe (1927) as chairman of the subcommittee on animal nutrition of the National Research Council, has asked research workers to assist in supplying analyses for manganese, fluorine, bromine, silicon, boron, aluminum, copper, arsenic and zinc.

Sherman in his Chemistry of Food and Nutrition (1926) comments upon manganese, zinc, copper and aluminum as possible normal constituents of the body. Bertrand has made extensive studies of the occurrence of nickel and cobalt in nature and believes them to be normal constitutents of animals as well as plant tissues. He has found both in the human body, nickel (1925) ranging from 0.09 mg. per kg. of fresh substance in the liver to 0.022 mg. in the brain; and cobalt (1925a) from 0.47 mg. per kg. of fresh substance in the spleen to 0.025 mg. in the muscles. From experiments on rabbits and dogs he believes that these elements are able to augment the hypoglycemic action of insulin. Osborne and Mendel (1913) attempting to secure growth of rats with diets containing "artificial protein-free milk" found that much better growth was secured when traces of iodine, manganese, fluorine and aluminum were added, although they were "without a guide as to the proper amount of these traces of inorganic elements to add." Daniels and Hutton (1925) finding that rats fed exclusively on cow's milk seldom reproduced, and that the deficiency appeared to be in mineral elements rather than vitamins, added to boiled milk from 7 to 10 gms. of soy bean powder per liter, with marked improvement in the reproductive capacity of the animals. The ash of the beans appeared to be equally effective. Analysis showed that silicon and aluminum were present. Milk contains naturally traces of aluminum, fluorine, and manganese, but it was thought these might be present in too small amounts. Accordingly a thick starch paste containing a mixture of aluminum potassium sulfate, sodium fluoride, sodium silicate and manganese sulfate was added to milk so that each animal received daily 1.5 mg. of each salt; and 1 drop of a 2 per cent solution of sodium iodide and 3 drops of a saturated solution of iron citrate were added to each 300 cc. of milk. On the milk thus supplemented the animals developed admirably and six generations were successfully produced.

Certain observers have suggested an association of elements such as copper, manganese and zinc with the vitamins because of their effect in increasing plant growth and their occurrence in the same part of the plant, e.g., in the bran of cereals (McHargue, 1925), but such a conclusion cannot be accepted without further experimental evidence. Because a mineral element is essential to a plant, it does not follow that it is indispensable for an animal. The importance of magnesium in the chlorophyl molecule, as shown by Willstätter or of copper as an essential in the structure of the hemocyanin which in certain marine organisms takes the place of the hemoglobin of other animals (Severy, 1923), cannot be adduced as good reasons for thinking these elements essential to human beings.

Flinn and Inouye (1928) have called attention to the fact that quantities of metals which cannot be tolerated by animals if given without food or drink in single doses, may be easily borne if mixed with the food eaten throughout the day. They found this especially true of copper. Rabbits died within 24 to 36 hours when 30 mg. of copper chloride solution was given by means of a stomach tube, but those taking 100 mg. each day with their food increased in weight and did not show ill effects over a period of months. They found in case of rats 98.5–99 per cent of copper, tin and nickel excreted when the animals were receiving 2 mg. of metal per rat per day. The rats at the beginning weighed about 75 gms. and grew at the same rate as the controls for a year.

There does not appear, then, to be any tendency to store these elements in a cumulative fashion. Whether the small amounts retained are signifícient has already been a matter of some investigation and the present status of our knowledge regarding aluminum, copper, manganese and zinc will be reviewed in some detail.

Aluminum

The wide distribution of aluminum in nature and the increasing use of this metal for cooking utensils from which small amounts may be dissolved by the food, especially if alkali or sodium chloride be present, has stimulated investigation as to its occurrence in foods and influence on the animal organism. Langworthy and Austin (1904), Myers and Voegtlin (1914), Gonnerman (1918) and Bertrand (1920) have reported significant amounts in common foods, including corn, sweet potatoes, parsnips, oatmeal, milk, butter and eggs. An estimate of the natural aluminum content of a seven day's diet for one adult person with a good variety of ordinary American foods made on the basis of these analyses showed a total of 3.3 gms. of the metal (Smith, 1928). The possible rôle of aluminum in nutrition has been recently discussed by McCollum, Raski and Becker (1928) who raised young rats on diets containing aluminum compounds. To a test diet consisting of yeast, 10 per cent; casein, 20 per cent; salt mixture, 4 per cent; agar, 2 per cent; butter fat, 8 per cent and dextrin, 56 per cent was added in one series 0.6 per cent of aluminum chloride and in another 3 per cent of a commercial brand of sodium aluminum sulfate, calcium acid phosphate baking powder which had been decomposed before combining with the test ration. This amount yielded approximately the same amount of aluminum as was present in the 0.6 per cent of aluminum chloride. There was no noticeable difference in a period of approximately six months between the

control rats and the aluminum-fed animals as regards growth, reproduction, or general well-being.

The above authors, using the Hilger quartz prism spectrograph were unable to find aluminum in a number of substances in which it had been reported and have said that "aluminum is not a constituent of either plant or animal matter." Kahlenberg and Closs (1929), however, have repeated these investigations using the same method and report aluminum present in egg, potato, carrot, English walnut meat, popcorn, lima, navy and lupin beans, peanut kernel, and lean beef. Wright and Papish (1929) report analyses of milk obtained from various parts of the United States and of Great Britain. The milk was milked straight into glass bottles and the ash studied spectroscopically. Among the metals found they include aluminum. In this situation, the most pertinent question would seem to be whether McCollum's control diet contained traces of aluminum sufficient for the growth of rats, since larger amounts apparently were harmless.

At any rate, there appears to be as yet no clear-cut answer to the question as to whether minute quantities of aluminum are essential to health and growth or not, but there is evidence that it has wide distribution in the tissues. Flynn and Inouye (1928) found in rats fed 2 mg. of aluminum daily for a year small amounts of the metal in heart, kidney, liver, lungs, spleen, and considerable in bone. Myers and Mull (1928,1928a) and Myers and Morrison (1928) have added further evidence of a small but fairly constant amount of aluminum in various tissues of the rat, the dog and man. In their work no attempt was made to secure an aluminum-free basal ration. In the rat experiments, animals on a normal stock diet were used as controls and to others on the same diet a daily portion of 2 mg. of aluminum per rat was given as potassium aluminum sulfate. Four generations were raised and a fifth generation weaned on this diet. Growth was normal and in excess of expectation as indicated by Donaldson's curve. Reproduction also appeared to be normal and the animals were healthy.

Analyses were made of various tissues in each group of animals, and also of some animals raised on an aluminum-free diet by Dr. Amy L. Daniels. In general those on the aluminum-free diet had the lowest amounts, those which were aluminum-fed the highest values, and the controls gave intermediate returns. The greatest difference between groups appeared to be in the livers, the aluminum-free averaging 0.08 gm. of aluminum per 100 gms. of tissue, the controls 0.14 gm. and

aluminum-fed, 0.19 gm. The increase in the tissues of the aluminum-fed rats were very small considering the amounts of aluminum fed.

In dogs fed a ration of table scraps, Myers and Morrison (1928) found aluminum present, and no marked increase in the amount in the tissues of others after daily ingestion of 0.23 gm. of aluminum (six dogs) or 1.55 gms. (2 dogs) for three months, with the exception of the livers, in which the amount averaged 0.27 gm. per 100 gms. of tissue in the aluminum-fed as compared with 0.15 mg. in the controls. It appears that when aluminum is administered orally little is held in the tissues.

Analyses of human tissues from hospital autopsy material (Myers and Mull, 1928a) showed aluminum in brain, heart, liver, gall-bladder and bile, kidney and spleen in concentrations quite comparable with the findings on the dog and rat, save that the proportion in the livers was somewhat lower, and in the brain and heart considerably higher.

There is, then, abundant evidence of the occurrence of aluminum in small amounts in food and in normal tissues, but practically no clear-cut evidence of any specific function. The aluminum appears to be held in the tissues for a considerable time when withdrawn from the diet, and if injected parenterally it is excreted very slowly. Feeding aluminum results in some increases in the amount in the body, most marked in the liver. It would seem that there is little absorption of aluminum from the alimentary tract. Flynn and Inouye (1928) found 70 per cent of aluminum administered to rats excreted in the feces and 30 per cent in the urine. Myers and Mull (1928) interpret the low percentage of aluminum in the tissues after prolonged aluminum feedings as indicating poor absorption.

Copper

The interesting fact that copper is an essential constituent of the blood of many marine animals has already been mentioned. It was first discovered in the blood of *Octopus vulgaris* and *Helix pomatia* by Harless in 1847, and the blue copper-containing protein of octopus blood, hemocyanin, was obtained by Fredericq in 1878. Two decades later it was prepared in crystalline form by Henze (1901) who noted its similarity in many respects to hemoglobin. Copper-containing proteins have been frequently studied and shown to occur in numerous gastropods and arthropods. The earlier literature has been reviewed by Vickery and Osborne (1928) and also by Severy (1923) who examined sea anemones, star fish, sea urchins, clams, mussels, abalone, shrimp, crab, salmon, etc.—fifteen different kinds of marine animals in all and found copper present in all but the clam and the whale in amounts varying from 0.564 mg. per kg.

in the sea-lion to 3.925 mg. in oysters and 13.07 in shrimp. Rose and Bodansky (1920) reported analyses of 20 common species of fish of which the average copper content was 2.5 mg. per kg. Although fishes have hemoglobin and are not dependent on hemocyanin as a carrier of oxygen, the authors believe copper persisting at a low level has biological significance. Bodansky (1921) found copper in the adult human brain to the extent of 3.6 to 6.0 mg. per kg.; since it was also present in one fetal brain in still larger amount (6.8 mg. per kg.), he concludes that there is more rapid storage in intrauterine life, as is the case with iron and iodine. Warburg and Krebs (1927) and Krebs (1928) have reported copper in human blood serum, as well as that of the dog, cat, rat, guinea pig, frog, chicken and goose. According to McHargue (1926, 1928) no product of either vegetable or animal matter has been found entirely free from copper. Bertrand (1920) and Supplee and Bellis (1922) agree that cow's milk averages 0.50–0.52 mg. of copper per kilo. Hess, Supplee and Bellis (1923) found commercially pasteurized milk to contain from 0.6–0.7 mg. per kg. The milk of one woman on a mixed diet which included a quart of milk a day yielded 0.40 mg. copper per liter and that of another 0.61 mg. It appears therefore, that infants whether fed human or cow's milk are constantly receiving small amounts of copper. A three-months-old infant observed for 7 days, excreted in the urine about 0.02 mg. of copper daily. Older infants up to 2 or 3 years of age excreted from 0.06 to 0.14 mg. per liter of urine. An adult on a dietary low in copper taken for two three-day periods, the urine being collected in each case for the last 36 hours, excreted 0.08 to 0.09 mg. of copper per liter, and another adult on a high copper diet excreted in similar periods 0.11 to 0.14 mg. of copper per liter. "Whatever may prove to be its physiological or pathological significance," say these authors, "our investigations show that cow's milk and woman's milk regularly contain copper, and furthermore, that in infants and adults copper is absorbed from the alimentary tract as proved from its constant presence in the urine."

Quam and Hellwig (1928) have extended the investigations on milk to other species and to processed milk and buttermilk. Raw cow's milk collected from four states contained from 0.37 to 0.50 mg. per liter; raw sheep's milk from 0.45 to 0.50 mg. per liter; raw goat's milk from 0.19 to 0.25 mg. per liter; pasteurized whole milk from 0.60 to 1.60 mgs. per liter; buttermilk from 2.40 to 2.50 mgs. per liter and four common brands of evaporated milk ranged from 1.80 to 2.70 per liter. In case of the processed milks the high copper content is due to the amount of copper absorbed from copper containers in the operation.

Lindow, Elvehjem and Peterson (1929) and Elvehjem and Hart (1929) have reported very extensive analyses of the copper content of plant and animal foods. The figures of Lindow, Elvehjem and Peterson range from 44.1 mgs. per kg. of fresh calf liver to 0.1 mg. per kg. of fresh celery. Per kilogram of fresh substance, 10 nuts average 11.6 mgs.; four dried legumes, 9.0 mgs.; nineteen cereals, 4.7 mgs.; eight dried fruits, 4.2 mgs.; four kinds of poultry 3.0 mgs.; seventeen kinds of fish, 2.5 mgs.; over thirteen animal tissues, 1.7 mgs.; two green legumes, 1.7 mgs.; eleven roots, tubers, stalks and bulbs, 1.4 mgs.; fourteen leafy vegetables, 1.2 mgs.; twenty-seven fresh fruits, 1.0 mg.; ten non-leafy vegetables, 0.7 mg.; oysters, chocolate, cocoa and molasses are strikingly high. The degree of variation in the copper content of foods falling in the same class was less than that of either manganese or iron.

Elvehjem and Hart (1929) found that lettuce grown on soil with copper added equivalent to 50 lbs. of $CuSO_4 \cdot 5H_2O$ per acre had a 25 per cent increase in copper content over that grown without the copper addition while that grown on soil with 500 lbs. to the acre had a 148 per cent increase. They find the copper content of feeding stuffs about one-fifth of the manganese and one-fifteenth of the iron content.

Flinn and Inouye (1928) fed rats for one year 2 mg. of copper per rat per day and found small amounts of the metal in all the tissues examined (brain, bones, hair, heart, kidney, liver, lungs, muscle and spleen). Aside from bones and hair the largest amount was in the liver but on the whole copper was retained only in small amounts. In domestic animals also it occurs in greater concentration in the liver than any other organ. McHargue, Healy and Hill (1928) found that the livers of calves at the time of birth contain more copper than do the livers of more mature animals of the same species. In order to find out whether the copper had any useful function, livers of young calves were dried, ground and analyzed, two equal portions of the moisture-free liver ashed separately and from one copper removed. The copper-containing liver ash was fed in small measured portions to a lot of rats which had been on a milk diet for four weeks and the copper-free ash similarly to a second lot of rats. At the end of a six-weeks period, hemoglobin determinations were made on the blood of the two lots of rats. The readings were distinctly higher in case of the rats which had received the copper. "From the external appearance of the rats, the color of the fresh blood together with the hemoglobin readings," the authors infer "that copper has an important function in the formation of hemoglobin and in the metabolism of animals having red blood."

Flinn and Von Glahn (1929) did not find the livers of workers in copper

industries higher in copper than those of human beings not so exposed (5 to 15 mg.); and rats, rabbits and guinea pigs fed copper daily for long periods showed no hemochromatosis in consequence. According to Flinn (1928) the blood of copper-fed pigs and dogs has an increased oxygen-carrying capacity over animals not fed copper.

In what form copper exists in plant and animal tissues is not known, but McHargue (1927) was able to demonstrate its presence in the ash of an extract of Kentucky blue grass made with hot 95 per cent alcohol and Hart and his associates (1928) found copper in the ash of a cold alcoholic extract of cabbage and lettuce.

Hart, Steenbock, Waddell and Elvehjem (1928a) having found the ash of beef liver, dried lettuce and yellow corn very potent in restoring the hemoglobin of the blood of rats with nutritional anemia to a normal level, whereas an equivalent amount of highly purified iron salts (0.5 mg. of iron 6 times per week) was ineffective, tried the addition of 0.25 mg. daily of copper as copper sulfate and 0.5 mg. of iron as ferric chloride to a whole milk diet. The response was immediate and striking. The hemoglobin rose from 2.68 gms. per 100 cc. of blood to 9.35 gms. in two weeks, and to 13.34 gms. in 6 weeks. "We think," say the authors, "that this is the first experiment in the literature giving to copper in association with iron the specific function of hemoglobin building in a mammal on an otherwise satisfactory diet." Other levels of copper feeding were then tried, from 0.01 mg. to 0.10 mg. per rat six times per week. The animals on the lowest dosage did not respond as quickly as the others, but in all the hemoglobin increased and there was general physical improvement as shown by better appetite, smoother hair, and greater activity.

In 1926, Minot and Murphy were able to show marked curative effects in pernicious anemia by the feeding of large amounts of fresh liver and in 1927 Cohn, Murphy and others found that the curative property resided in a fraction representing the non-protein extractions. They made a preparation free from lecithin and other ether-soluble substances, and from all but a trace of iron and sulfur. This preparation when used by Hart and his associates was not effective in raising the hemoglobin of the blood of anemic rats, unless the animals received additional iron, but the response was very rapid when the iron was given. Actual determinations of the copper content of the ash of the liver extract revealed 0.016 per cent of copper; consequently along with 0.5 mg. of iron the rats were receiving 0.05 mg. of copper daily.

The work of Hart and his coworkers was very shortly confirmed by McHargue, Healy and Hill (1928), whose work on copper in relation to

hemoglobin formation has already been referred to. Elden, Sperry Robscheit-Robbins and Whipple (1928), who had found the ash of apricots, liver and kidney potent for the cure of anemia produced in dogs by bleeding have reported the finding of copper in the ash in each instance. However, administration to anemic dogs of copper sulfate in doses equivalent to 5 to 65 mg. of copper daily did not result in a large increase of new red cells and hemoglobin, nor was copper tartrate in daily doses, equivalent to 40 to 60 mg. of copper more potent than copper sulfate. The original ash of the food material was more effective than either, and the authors conclude that a balanced salt mixture and not copper alone is essential to hemoglobin regeneration in dogs.

Drabkin and Waggoner (1929) have been able to maintain dogs for from six to 18 months on a diet containing 0.052 mg. of copper per kilogram of body weight (or only about one fourth as much copper as Hart and his associates supplied to rats exclusively milk-fed) without apparently any lowering of the hemoglobin level or any diminution in speed of recovery after single large bleedings. These investigators, like Whipple and his associates, observed no improvement upon increase of copper to 1.3 mg. per kilogram of body weight. They also prepared a copper-free synthetic diet for young rats, using coagulated egg albumin instead of commercial casein and dry brewer's yeast as a source of the vitamin B complex, as commercial casein and vitavose were found to contain copper. Rats made anemic by means of a whole milk diet recovered when placed on this copper-free ration. How this is to be interpreted in light of the work of Hart and his associates is not clear at the present time for on the whole the evidence indicates that copper, so widely distributed in the animal body and so definitely a constitutent of the liver, must be regarded as an element of importance in iron metabolism. Further developments are awaited with great interest.

Manganese

Manganese appears to be present in most living tissues both animal and vegetable. It is most abundant in those parts of plants which are most active—young leaves and shoots and seeds; in the latter its presence seems to be necessary to the development of the young plant (Bertrand and Rosenblatt, 1921,1922). In the animal body it is widely distributed but the largest amounts appear to be in the liver, pancreas, lymph nodes and kidney, while muscles, lungs and liver contain very little. In the blood it is present in fairly constant concentration.

Reiman and Minot (1920) have reviewed the literature regarding manganese in the blood and tissues of man and animals. Analyses of

human tissues gave the following average amounts per 100 grams of fresh substance: liver, 0.170 mg.; pancreas, 0.076 mg.; lymph nodes, 0.063 mg.; kidney, 0.061 mg.; and stomach, small intestine, colon, adrenals lung, muscle, heart and brain from 0.033 to 0.013 mg.; blood from 0.004 mg. to 0.020 mg.

The literature regarding manganese in plant tissues has been reviewed by Lindow and Peterson (1927) who report analyses of 84 samples covering the principle classes of human foods. Those having from 100 to 200 mg. per kg. of dry material are beet tops, blueberries, lettuce, pineapple and wheat bran; with from 35 to 100 mg. per kg., beets, blackberries, chard, oats, spinach and wheat (whole grain); fruits as a rule have less than 15 mg. per kg., and round of beef and a number of fish examined have little or none. Variations in plant material are wide, due presumably to the nature of the soil in which they are grown. This is emphasized by analyses of animal feeding-stuffs made by Skinner and Peterson (1928).

According to Walbum and Mörch (1923) manganese increased the antitoxic power of horse serum if injected into the horse in daily doses of 25 cc. of N/100 manganese chloride.

McCarrison (1927a) noted the relative richness of wheat in manganese in comparison with rice paddy and other food grains of India and experimented with two groups of rats, one receiving 0.56 mg. of manganese daily, the other 0.009 mg. The few animals in both groups were apparently in good health and were comparable as to food consumption; although the group with the larger dosage did not grow quite as well as the controls on an adequate diet without the manganese added, and the others grew slightly better than the controls, the differences scarcely seem significant, especially as the basal diet itself contained traces of manganese.

Levine and Sohm (1924) who gave young rats an adequate diet and added to their drinking water manganese sulfate in dilutions of 1:2000, 1:4000, 1:10,000, reported that the manganese-fed animals were livelier and sleeker and grew better than the controls, and their offspring grew somewhat faster than the average, but the differences cannot be regarded as striking.

McHargue (1926) fed young rats a basal ration to which manganese or manganese in conjunction with copper and zinc were fed for weeks, but owing to lack of vitamins in the basal ration they all declined rapidly, and it hardly seems fair to attribute any difference in rate to the added mineral elements, since in groups of three or four animals such differences commonly occur owing to difference of the vitamin reserves of the animals or in the length of time in which these are exhausted.

A comparison of the influence of copper and manganese on the hemoglobin of the blood of rats made anemic by whole milk feedings has been made by Titus, Cave and Hughes (1928)who found accidentally that rats grew anemic with some samples of Fe_2O_3 as the sole source of iron and failed to do so with others. Investigation showed that the samples which prevented anemia contained considerable manganese. In their series of experiments with three groups of ten rats each, there was a difference in the speed with which the anemic animals recovered, those receiving manganese in addition to copper doing so most rapidly. Those receiving only manganese also responded fairly well, but not as well as those receiving copper as the only supplement to a milk-iron ration.

From the foregoing summary of our knowledge regarding manganese it is apparent that its occurrence in nature is widespread, but that as yet the evidence of specific functions in the animal organism is exceedingly meager.

Zinc

The occurrence of zinc in plant and animal matter seems as general as that of aluminum, copper and manganese. In fact, association of the latter three has been commonly remarked by the workers in this field. The literature regarding the distribution of zinc has been exhaustively reviewed by Lutz (1926). It is present in sea water and often in spring and mineral waters, in the latter varying from 0.03 to 8.0 mg. per kilo. Soils in general contain small amounts, but in many localities the concentration is fairly high. Zinc has been found in molds and algae and in all the organs of phanerogamous plants. It was first reported in foods in 1877, and researches from that time to this regarding vegetable foods have been summarized by Bertrand and Benson (1928), who found the pulp of peaches, plums and apricots to yield less than 1 mg. per kg., while carrots, turnips, radishes, orange pulp, lemon juce, and leaves low in chlorophyll, as cabbage and romaine, ranged from 1 to 2 mg. per kilo. Celery, bananas, cauliflower, salsify, sweet potatoes, tomatoes and Jerusalem artichoke contained from 2 to 3 mg. per kg. and lettuce, cress, spinach and dandelion leaves from 4.5 to 10 mg. per kilo. Four dried seeds of legumes yielded from 20 to 50 mg. per kilo.

Lutz (1926) believes that zinc is an integral part of the plant cell and points out its occurrence in commercial corn starch (35 mg. per kg.), flour (8 mg. per kg.), with even traces in soluble starch. He reports the zinc content of grains as ranging from 14 to 85 mg. per kg., the range of wheat being from 26 to 85 mg. per kg., other cereals less. In grains the metal

is concentrated in the outer coats, wheat bran yielding as much as 139 mg. per kilo.

The presence of zinc in the liver of *Sycotypus canaliculatus*, a large, carnivorous snail of Long Island Sound, and in *Fulgur carica*, an allied mollusk, was discovered by Bradley (1904). Subsequently, Mendel and Bradley (1905) found the zinc in *Sycotypus* limited to the blood and liver, and showed (1906–07) that it is an essential constituent of the respiratory protein of this mollusk, analagous to the hemocyanins, and accordingly named by them "hemosycotypin." They expressed the belief that other marine forms would be found to contain zinc, since it is present in sea water, and such has proven to be the case. Marine animals have been analyzed for zinc by a number of investigators, among whom special mention may be made of Bodansky (1920), who studied twenty species from the Atlantic coast, and Severy (1923), who made a similar study of forms on the Pacific coast. Both of these workers found the oyster to have the highest zinc content of all the animals examined, but the western oyster had only about one-fourth as much as the eastern, the latter ranging from 188.5 to 341 mg. per kg. of fresh substance according to Bodansky, who also reports that this zinc is rather evenly distributed in the digestive organs, mantle, and gills, with a much lower percentage in the muscle. Severy investigated the distribution of zinc in the *Cryptochiton* (*stelleri*) and found that most of the metal was located in the mantle and muscle. In the sea lion and whale, both of which were relatively high in zinc, though less than half as high as the western oyster, from 80 to 90 per cent of the total was found in liver and muscle, divided almost equally between the two.

Birckner (1919) reported zinc in the yolk of hen's eggs, amounting to about 0.005 per cent of the fresh substance, or estimated as about 1 mg. per egg. Mixed market milk (12 samples) averaged 4.2 mg. per kg., and samples of human milk from three negro women all showed a higher concentration of zinc than cow's milk.

In the human body zinc is widely distributed and Lutz estimates the total amount in the body of a 70 kg. man as about 2.2 gms.—nearly as much as the amount of iron, as estimated by Sherman (1926), 2.8 gms. The largest amounts were found in the bones and hair, then in decreasing quantities in liver, kidney, and muscles; heart, pancreas, spleen, small and large intestines, stomach and ovary; testes, adrenals, brain and lungs.

The first attempts to determine whether zinc is essential for the growth of animals were made by Bertrand and Benson (1922) and McHargue (1924), but as neither of the experimental diets contained adequate vita-

mins for growth, these workers had no evidence of the effect of zinc except the survival period of the animals, and as in both cases the number of animals was very small, and the differences from group to group no greater than one often sees with vitamin deficiencies, it is impossible to draw any conclusions from them. Bertrand and Benson took care to use as a control a diet free from zinc, but McHargue simply superimposed additional zinc upon a ration already containing some.

Long-continued ingestion of zinc by cats, dogs, and rats was found by Drinker, Thompson and Marsh (1927) to increase to a limited degree the concentration of the metal in the body. For periods of three to 33 weeks cats and dogs were fed daily doses ranging from 175 to 1000 mg. of zinc oxide without any apparent ill effects. Rats given from 2 to 38 mg. of zinc per day as oxide grew normally and their offspring, successfully reared, also grew to normal size. The concentration was from 0.038 to 0.040 mg. of zinc per gram of tissue, maintained quite consistently regardless of age. This is in essential agreement with the work of Fairhall (1926), who found that rats receiving 0.48 mg. of zinc daily stored on the average 0.04 mg. per day. Fairhall also studied the zinc excretion of a man on an ordinary mixed diet and found the average excretion in the urine to be one mg. per day but the fecal zinc varied widely, the quantity being directly influenced by the amount ingested. Drinker, Fehnel and Marsh (1927) have reviewed the literature on this subject, and have reported findings with regard to the zinc elimination of man in harmony with Fairhall's observation, the urinary output in their similar experiments ranging from 0.25 to 2.0 mg. (average 0.89 mg.) and the fecal output varying with the diet.

Special investigations of the alleged toxicity of zinc by Batchelor, Fehnel, Thompson and Drinker (1926) and by Heller and Burke (1927) furnish additional evidence that if zinc is ingested it is again excreted, as large storage in internal organs has been in no instance found.

The rôle of zinc in normal nutrition has been discussed by Hubbell and Mendel (1927), who tried to prepare a diet for mice adequate in vitamins and free from zinc. This proved to be impossible, but they did succeed in making one containing only 0.014 mg. per gm. of food, giving a mouse a daily intake of 0.005 mg. of zinc. On this diet there was a slight but definite retardation of growth, but when to the zinc-low diet was added zinc sulfate to make 0.02 mg. of zinc per mouse per day, there was slightly better growth. Doubling this latter amount seems to have resulted in no advantage to the males, but another slight increment of gain for the females. It is interesting to note that control animals,

fed on a diet of casein, cornstarch, crisco, cod liver oil, salt mixture and and yeast powder had a zinc intake of approximately 0.318 mg. per day, or eight times as much as the experimental animals on the highest dosage. The animals on the zinc-low diet showed a slight loss of body zinc, and the authors conclude: "It is not unlikely however, that there is some variation in growth with varying amounts of zinc and that the metal is not merely an accidental factor in the nutrition of the mouse."

M. S. R

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